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TECHNICAL

# SPORE GERMINATIONS OF CEREAL SMUTS

BY

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## LETTER OF TRANSMITTAL

*University Farm, St. Paul, Minn., April 23, 1912*

A. F. Woods, *Director,*

*Minnesota Agricultural Experiment Station.*

Dear Sir: This bulletin contains a historical summary of the development of knowledge concerning the identity of the various cereal smuts. Synonymies of the various forms are also given.

Original observations, illustrated by text-figures, are made on the morphological features of spore germination in the various forms. The effect of freezing is noted, and the correlation between the characteristics of spore germination and infection phenomena are shown. The bulletin is intended principally for students and investigators.

I recommend that this be published as a technical bulletin of this Station.

Respectfully submitted,

E. M. Freeman

*Chief of Division of Plant Pathology and Botany*

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## ILLUSTRATIONS

# SPORE GERMINATIONS OF CEREAL SMUTS*

## INTRODUCTION

The study of smuts began with the ancients. In the first century Pliny (1535, Translation 1890, vol. 4, p. 54), in his *Naturalis Historiae,* speaks of the smuts and discusses the effects of weather and location on their prevalence. Dodoens (1578, p. 471) says that smut occurs on oats and wheat, mainly on oats. He speaks of it as a barren and unfruitful herb—*Ustilago,* blackened or blighted. He adds that it is worst when the sun is hot after a rain. Theophrastus (1644, p. 499) speaks of the smuts, but, of course, knew little of their true nature. The idea commonly accepted was that the smut was an abnormality of the plant itself, the plant merely being sick in some functional way. Persoon (1801, p. 224) first gave the smuts a definite place among the fungi; while Prevost (1807, p. 29) showed that the spores, which had been considered abnormal cells of the plant, were capable of germination. Many diverse opinions were still held, however. Fries (1829, p. 504) regarded the smut-mass as a product of the host plant; Unger (1833, p. 356), however, thought it was a sickness. De Candolle (1832, p. 1435) understood the real nature of the parasites, and the work of Tulasne (1847, 1854) was epoch-making. He worked on the spore germinations, observing the germination phenomena of both *Tilletia* and *Ustilago* forms. He used the term "promycelium" for the first tube sent out by the spores, and designated secondary spores as "sporidia" (1854, p. 159). Léveillé (1839), DeBary (1863), Fischer von Waldheim (1870), and Hoffmann (1860, p. 273) all contributed to the knowledge of the biology of the smuts. Much was learned concerning the spore structure and the development of the mycelium from the spores, the methods of infection, and the development of the spores. However, the so-called loose smuts of barley, wheat, and oats were still included in the same species under the names of either *Ustilago segetum* (Bull.) Ditt. or *Ustilago carbo* (B. C.) Tul. Brefeld (1883, p. 1) showed that smuts could be propagated saprophytically. He began very extensive experiments with artificial cultures of smut fungi, observing especially the germination phenomena of spores. As a result of these observations Brefeld found, among other things, that the spores of a barley smut which he had received from Japan germinated

*The author takes pleasure in making acknowledgment to Mr. E. C. Johnson, formerly Cereal Pathologist of the Bureau of Plant Industry, and Mr. A. A. Potter, Assistant Pathologist of the Office of Grain Investigations, for suggestions and criticisms and in particular to Dr. E. M. Freeman, under whom the work was done.

differently from those which he had previously observed (1888, p. 1590). He found that the smut spores from Japan produced no conidia, whereas those collected in Germany had produced conidia in great abundance. On trying some spores of barley smut from Germany in order to make comparison, he found that they always germinated without producing conidia. He also found the same to be true in the case of "Flugbrand" of wheat. He therefore concluded that the smut of barley and wheat was not the same as that on oats. He named the new smut *Ustilago hordei.* This species, then, according to Brefeld, included the "Flugbrand" on both wheat and barley.

Jensen (1888b, p. 405) about the same time undertook to determine whether or not the smut which attacked barley, oats, and wheat was caused by the same fungus. He conducted cross-inoculation experiments with smut from the three forms. His method was to dust spores on the kernels. He found that smutted plants resulted only from dusting each kind of seed grain with spores from that particular kind of grain. He therefore concluded that the smuts on the different hosts were at least different varieties and intimated that they might be different species. He got only a fraction of one per cent of infection in the case of loose smut on wheat. He explained this by saying that the spores of wheat smut retained their vitality for a much shorter time than those of the other forms. The real reason was not known until floral infection was demonstrated a number of years later. Therefore, Jensen's separation of the two loose smuts, that on wheat from that on barley, in so far as it rested on the basis of infection experiments, was at that time unwarranted.

In addition to the work above mentioned, Jensen also showed that there were two distinct barley smuts. These he separated on the basis of the disintegration of the spore masses. The smut with a powdery spore-mass, early blown away, he called *Ustilago segetum* var. *hordei nuda.* The other, in which the spore-mass was harder and more persistent, he designated *Ustilago segetum* var. *tecta.*

Rostrup (1890, p. 17) later showed that one of the barley smuts, var. *tecta* of Jensen, produced smutted grain when the spores were dusted on the seed, while the other one, var. *nuda* of Jensen, did not. The first he calls *Ustilago Jensenii* n. sp., while for the latter he retains Brefeld's name, *Ustilago hordei.* The following is Jensen's classification of the loose smuts:

Wheat smut, *Ustilago segetum,* var. *tritici.*
Oat smut, *Ustilago segetum,* var. *avenae.*
Naked barley smut, *Ustilago segetum,* var. *hordei nuda.*
Covered barley smut, *Ustilago segetum,* var. *hordei tecta.*

He also adds that some smutted ears of oats are quite different from others, but gives no details.

Kellerman and Swingle (1890, p. 213), after carefully working out the germination phenomena of spores of the various loose smuts, separated them into four species, and one of these species, that on oats, was found to have a variety. The loose smut of wheat they called *Ustilago tritici;* that of barley, *Ustilago nuda;* oat smut, *Ustilago avenae;* and covered barley smut, *Ustilago hordei.* The variety of *Ustilago avenae* they distinguished on account of the darker spore-mass and smooth spore wall. It was designated *Ustilago avenae* var. *levis.* It was found that of these the first two never produced conidia, whereas the others often produced them in great abundance. On account of this fact Herzberg (1895, p. 7) suggested that the forms producing no conidia be given the generic name *Ustilagidium.*

It was found by Maddox (1895, p. 2) that both *Ustilago tritici* and *Ustilago nuda* infected the young ovaries at flowering time. He did not show the histological details. This was done for barley by Hecke (1905, p. 248), who traced the mycelium into the growing point of the embryo. Hori (1907, p. 165) and Brefeld (1903) had also concluded that floral infection took place.

It will thus be seen that floral infection, as far as the common cereal smuts are concerned, is correlated with the absence of the production of conidia in the germination of spores.

The histories of *Ustilago zeae* and of *Tilletia foetens* are given before the original observations on them, and are not included here, since they were early separated from the so-called smut forms.

The smuts considered in this paper are: loose smut of wheat, *Ustilago tritici* (Pers.) Jens.; loose smut of barley, *Ustilago nuda* (Jens.) Kell. and Sw.; covered smut, stinking smut, or bunt of wheat, *Tilletia foetens* (B. and C.) Trel.; covered smut of barley, *Ustilago hordei* (Pers.) Kell. and Sw.; smut of oats, *Ustilago avenae* (Pers.) Jens., and maize smut, *Ustilago zeae* (Beckm.) Unger.

Although a great deal of work had been done on the germination phenomena of smut spores, it was thought that a careful study might contribute knowledge which would be of some value, especially in explaining infection.

## LOOSE SMUT OF WHEAT

### *Ustilago tritici* (Pers.) Rostr.

#### HISTORY

As has been mentioned before, all the loose smuts were originally considered the same; so *Ustilago tritici* was first known as *Ustilago*

*segetum* and had a number of names before the present one was finally applied. The smut was known as early as 1552, when Bock (1552, p. 666) called it Ustilago, as he did not recognize it as a fungus. Bauhin (1623, pp. 23, 24) named it *Ustilago secalena;* but he, also, failed to recognize it as a fungus.

It was not until much later that the so-called "brand" was recognized as a parasitic plant. Various names were applied. Persoon (1801, p. 224) called it *Uredo tritici,* a variety of *Uredo segetum;* while De Candolle (1815, p. 75) considered it a variety of *Ustilago carbo,* as did Tulasne (1847, p. 80) who named it *Ustilago carbo* a *vulgaris* a *Triticea.* Wallroth (1833, p. 217) applied still another name, *Erysibe vera tritici.* None of these men observed any fundamental difference between the form on wheat and those forms found on oats and barley.

Jensen (1888b, p. 405) in 1888 attempted to show the individuality of these various forms. He conducted cross-inoculation experiments with wheat, oat, and barley smuts. In dusting spores from wheat, oat, and barley smuts on wheat kernels, he found that only those plants which had been dusted with spores from smut found on wheat produced smutted heads. His percentage of smutted heads in wheat was, of course, low, the reason for this being evident in the light of later experiments and the discovery of intraseminal infection of loose smut of wheat. Jensen also asserted that spores of wheat smut retained their vitality a much shorter time than those of most other forms. Consequently he said that wheat was much less liable to be smutted than other cereals.

Plowright (1889, p. 70) noticed that *Ustilago segetum,* when found on wheat, had a decided golden luster; while on *Avena elatior* he observed that it was sooty black. He predicted that careful physiological research would, in the future, show two distinct species. Fischer von Waldheim (1870, p. 63), in studying the germination of various smut spores, noted the greater length of promycelia of wheat smut spores, and also maintained that the promycelium disorganized earlier in this form than in those from either oats or barley, often within four days. Kellerman and Swingle's (l. c. pp. 261-267) studies of germination, in 1888, showed clearly that the loose smut of wheat was a distinct species. It was named *Ustilago tritici* (Pers.) Rostr.

The following is a synonomy of the species:

Ustilago. Bock, De stirpium. Historia Commentarius: 666. 1552.

Ustilago secalena. Bauhin, Phytopinax: 52. 1596.

Uredo segetum b. Tritici. Persoon, Tentamen dispositionis methodicae fungorum: 56-57. 1797.

Uredo (Ustilago) segetum b. Tritici. Persoon, Synopsis methodica fungorum: 224. 1801.

Caeoma D. Ustilago. Link, Observationes in Ordines plantarum naturales—I. Magazin für die Neuesten Entdeckungen in der Gesammten Naturkunde der Gesellschaft Naturforschender Freunde zu Berlin **3**:6. 1809.

Uredo carbo b. Tritici. De Candolle, Flore française **5**:76. 1815.

Ustilago segetum. Ditmar in Sturm, Deutchlands flora III. **1**:67. 1817.

Caeoma segetum. Link, in Linné, Species Plantarum **6**$^2$:1. 1825. (ed. Willd.)

Erysibe vera b. Tritici. Wallroth, Flora Cryptogamica Germaniae II. **4**:217. 1833.

Uredo carbo Tritici. Philippar, Traité sur la carie: 92. 1837.

Ustilago Carbo, a. vulgaris a. Triticea. L. R. et Ch. Tulasne, Mémoire sur les Ustilaginées comparées aux les Uredinées. Ann. Sci. Nat. Bot. III. **7**:80. 1847.

Ustilago Hordei. Brefeld, Neue Untersuchungen über die Brandpilze und Brandkrankheiten II. Nachrichten aus dem Klub der Landwirthe zu Berlin 221:1593. 28 Je. 1888.

Ustilago segetum var. tritici. Jensen, Propagation and Prevention of Smut in Oats and Barley. Jour. Roy. Agr. Soc. Eng. **24**$^2$:407. 1888.

Ustilago Tritici. Jensen in letter to Kellerman and Swingle, January, 1890. (According to Kellerman and Swingle: 262. See below.)

Ustilago Tritici (Pers.) Jensen. Kellerman and Swingle, Ann. Rep. Kan. Agr. Exp. Sta. **2**:262. 1890.

Ustilago Tritici (Pers.) Rostrup, Overs. K. Danske Vid. Selsk. Forh. **1890**:15. 1890.

Ustilago Tritici (Pers.) Jens. forma folicola. P. Hennings, Zeits. Pflanzenk. **4**:139. 1894.

Ustilagidium Tritici. Herzberg, in Zopf, Beitr. Phys. Morph. Org. 5:7, 1895.

## METHODS EMPLOYED IN GERMINATION TESTS

Spores of all the forms were collected as soon as ripe, in the summer of 1909. Some were kept at room temperature, dry, and in the dark; others were partly buried in pots and placed outside in order that they might be exposed to winter conditions in the soil; while still others were put in petri dishes and these placed outside where the spores would be exposed to both direct sunlight and winter conditions. Some were also put in petri dishes and kept at room temperature, but were exposed to direct sunlight. All germinations described, except where otherwise specified, were obtained at room temperature from spores which had been kept dry in the dark in the laboratory.

For all germinations, both of this and the other forms, various media were tried. Tap water, distilled water, and nutrient solutions were used for purposes of comparison. For all careful work, the Marshall Ward cell was used; while for comparison, germination tests were also made in watch crystals. Tests were made at intervals of a few weeks, beginning in July, 1909, and continuing until March, 1911.

## DESCRIPTION OF SPORES OF USTILAGO TRITICI

The spores of *Ustilago tritici* are oval, subglobose, or globose, variable both in shape and size. The shape is probably more often subglobose, or even globose, but not infrequently more elongate. The color is olive-brown to golden, often with a bronze tint. One side of the spore is conspicuously lighter in color than the other, the lighter side being not infrequently almost hyaline. The wall on this side is usually thinner than on the darker side. The spore wall consists of two distinct coats, the outer, or epispore, and the inner, or endospore. The dividing line in optical section between epispore and endospore is sometimes difficult to see on account of the fact that the endospore is usually hyaline. When both are visible in optical section, it is usually on the darker side of the spore, where the contrast between the dark coloration and the hyaline endospore is more marked than on the lighter side. By the use of potassium hydroxide, the two parts of the wall may be quite readily distinguished. Sometimes it is possible to trace the line of demarcation around the entire spore. The epispore is practically always echinulate, sometimes minutely and sometimes quite distinctly so. In all cases the warty excrescences show only on the lighter-colored side, sometimes only along the edge in optical section, and in other cases covering about one half of the spore (Plate I, *a*). In size the spores vary considerably. They measure from 4.75 to 9.5 μ in diameter, averaging about 5.5 to 7.5 by 5 to 6 μ.

## GERMINATION OF SPORES OF USTILAGO TRITICI IN WATER

The spores do not germinate as readily as those of some other species, especially *Ustilago avenae*, *Ustilago hordei,* and even *Ustilago nuda.* It requires from 14 to 17 hours for them visibly to begin germination.

Germination proceeds always, as far as could be observed, from the light-colored area of the spore. In a few cases the promycelium comes out from the very edge of this region, but in no case was it seen to come from the dark side. Very rarely is the spore wall split by the young promycelium. In fact there is a definite pore through which the promycelial tube comes. Normally only one promycelium is produced, but in some cases germination proceeds from two places. When this occurs, there are usually two light-colored areas and the promycelia grow out from these.

The promycelium is usually constricted at the base, just where it emerges (Plate I, *a* to *k*), and sometimes has a swollen tip (Plate I, *e, h, i;* Plate VII, *a* to *d*). It may be either curved or straight, at first about 35 μ long and 4 μ in diameter. Before 20 hours the septations

are usually quite indistinct, sometimes none being visible. Some of the promycelia are one- or two-septate, while others are continuous. At this time there are practically no vacuoles in the protoplasm, which is still very finely granular.

In 22 hours the promycelia have ordinarily reached a length of from 30 to 40 μ, and are very clearly from 1- to 5-septate, usually 2- or 3-septate. As yet there are normally no branches, or only a few just beginning to form, and vacuoles in the protoplasm are just beginning to appear. In 24 hours branching has begun, and in some cases vacuoles are already quite conspicuous (Plate I). A few fusions have sometimes taken place by this time. Usually these are between branches of the same promycelium, or between the promycelium itself and one of its branches. In some cases tubes from different spores fuse, and a single promycelium is formed above the fusion, but this is seen infrequently (Plate II, *h*). So-called "knee-joint" fusions, appearing often merely as slight protuberances, are fairly common. These knee-joint fusions occur as a result of the outgrowth of two short, stubby branches, one on each side of a septum. These branches grow together and produce a knoblike protuberance, which, later on, as the cells swell up, may open and appear as though a tube had grown out from one cell and fused with that next to it (Plate II, *a*).

At from 25 to 27 hours, or, quite often, before this time, the promycelia have attained practically their full size. When fully grown, they are of various forms and sizes. Some are straight, unbranched, and 2- to 3-septate, while others are quite profusely branched, irregular in shape, and many-septate. The branches arise most often just below a septum and are usually curved. They may in turn branch; but, until after the expiration of four or five days, this does not usually occur to a very great extent.

After the promycelium itself has ceased growth, it sends out germ tubes or infection threads. These arise first either as branches or as continuations of the promycelium, differing in no essential particular from ordinary branches. As they grow out, the basal portion becomes first vacuolated and then vacant. As the protoplasm goes forward, leaving behind nothing but the walls, with sometimes a thin film of protoplasm, septations appear, so that there is a basal, segmented, vacant portion, and a swollen tip filled with protoplasm. Sometimes more than one germ tube is sent out from the same promycelium, usually when the promycelial tube itself grows out and also sends out a branch. However, the branching may not occur until the germ tube is already well formed and grown to a considerable length. In the earlier stages the promycelial cells retain their protoplasm, even after

the bases of the germ tubes have become entirely vacant. The promycelium may be well supplied with protoplasm, while growing out from it there is an infection thread with a vacant basal portion very often much longer than the promycelium itself. This condition does not last long, however. The promycelial cells soon lose their protoplasm also. Very often the first cells to become vacant are the basal ones, but this is not necessarily the case. In 96 hours nearly all of the original promycelia are entirely vacant and the germ tube seems to come directly from the spore. There are some exceptions to this, one of which is shown in Plate III, *c*.

These germ tubes may branch; in fact they quite often do. Usually branching takes place near the tip. When branches are sent out, they are protoplasmic throughout at first. Sometimes, however, in a comparatively short time they become very much like the original germ thread. Fusions occur either with branches from the same filament or with those from others, so that sometimes a network is formed. This is rarer in water, however, than in nutrient solutions. A number of these cases of branching and fusions are shown in Plates II and III. More often the germ threads appear as do those shown in Plate III. They are long and narrow, usually 1 to 2 μ wide in the basal portion, and about 2 to 3½ μ wide in the swollen tip. After 4½ days there was but little change. The tubes were fully grown and reached a length of 300 to 400 μ, the tip, not always continuous, measuring about 30 to 90 by 3 to 4 μ, and the vacant, segmented, basal portion about 1 to 2½ μ.

### SUMMARY OF GERMINATION OF SPORES OF USTILAGO TRITICI IN WATER

Germination in water takes place in about 14 or 15 hours, although sometimes it requires a longer time. A promycelium having from 2 to 5 septa or sometimes more and about 30 to 40 μ long and from 3 to 4 μ wide, is sent out from the lighter-colored portion of the spore. This branches in some cases, but produces neither sporidia nor free segments, remains attached to the spore, and sends out very long, narrow germ tubes or infection threads, which consist of a long, segmented, basal portion and a swollen, protoplasmic tip which may or may not branch. Fusions sometimes take place, especially "knee joints," from which germ tubes often grow.

### GERMINATION OF SPORES OF USTILAGO TRITICI IN NUTRIENT SOLUTION

Of the nutrient media used, a 5 per cent solution of sugar seemed most favorable, and the following description is of spores germinating in that medium.

The early stages are very nearly the same as those just described for germinations in water. The growth of promycelia proceeds more vigorously, but in essential characters, such as branching, fusions, etc., there is no important difference. The promycelium has from 2 to 5 septa or, in rare cases, even more. It may be branched or unbranched, often constricted, and sometimes vacant at the base.

Well-marked differences appear after about 20 hours. The septations are much more distinct, and the cells of the promycelium seem much less firmly united than those in water; in fact there was a distinct tendency for the promycelium to break up into component cells. This tendency seemed especially strong when the spores were still fresh. Spores collected in July, 1909, were immediately tried in watch crystals and Ward cells. Germination took place within 14 hours, and the promycelia formed very quickly. The tendency to break up, however, was quite pronounced. In some cases the entire promycelium became detached from the spore and then broke up into segments; in other instances the end cells were freed, while the others remained attached. These free cells were very closely watched, but none of them divided again; nor did any send out tubes. Spores collected at the same time as those whose germination was studied in July were tried in March. They did not form free segments, although in other respects they resembled the others quite closely.

The later stages are somewhat different from those in water. Germ tubes, such as those previously described, are sent out in great abundance. They are of the same general character as those produced in water, except for the fact that they are generally thicker, and the swollen tips are more coarsely granular (Plates IV and V). There also seems to be a greater tendency toward branching in the nutrient solution. At 10 days the tips, which by this time were swollen to a thickness of 4 or 5 μ and were very coarsely granular, had sent out many septate or non-septate branches, a majority of which were quite short and blunt. The typical appearance of the tips at this stage is shown in Plate V. As will be noticed from the figures, the tips are very often irregularly septate, long and short, thick and narrow cells alternating in no definite manner. Quite frequently the tip sent out long, irregular tubes, often non-septate and resembling primary branches in most respects except for their irregular shape.

Beginning about the twelfth day, or even before that, the tips of the germ tubes, now from 75 to 100 μ long, began to form very definite septa which often occurred at quite regular intervals. The swelling continued, the diameter of the segments being not uncommonly 6 or even 7 μ. This process continued until at 25 days nearly all

of the tips were of this nature (Plate IV, *a* to *h*). At the same time the vacant segments of the germ tube, and even the basal cells of the filled tip in some cases disintegrated and left chains of cells such as those shown in Plate VI, *g*. Forms such as that shown at *e* in the same plate were very common; in fact by this time nearly all of the tips showed a structure very closely resembling in all essentials the drawings in this plate. By 27 days the nourishment was practically exhausted and the cell had begun to dry up. A large percentage of the chains resembling the one shown in Plate VI, *f* had now divided at the septa, leaving the individual segments entirely free from each other, as in Plate VI, *h* and *j*. These segments had now become more nearly spherical with a diameter of from 6 to 9 $\mu$, being about the size of the original spore. The wall thickened until it could be plainly distinguished even while the segments were still grayish in color. This wall became thicker, while the segments, at first grayish in color, now assumed an olive hue or a somewhat golden luster. Except in a few cases the segments were neither as dark as the original spores nor as distinctly echinulate in appearance, but in size, shape, and color they resembled them very closely.

The cell was now allowed to dry out completely. Then more sugar solution was added and the cell placed under the same conditions as at first. Within 16 hours the rounded segments sent out tubes resembling very closely the promycelial tubes of the original spore. It is probable that, owing to the exhaustion of nourishment, the protoplasmic contents contracted and formed resting cells which appeared very much like the original spores. The culture was kept for 42 days and by that time a large percentage of the segments had sent out tubes.

### SUMMARY OF GERMINATION OF SPORES OF USTILAGO TRITICI IN SUGAR SOLUTION

The germination in sugar solution proceeds very much as in water in the earlier stages. However, as contrasted with germinations in water, in some cultures free segments were formed within 48 hours by the breaking up of the promycelium. The growth of promycelium and branches was more vigorous than in water and fusions were probably more frequent. The germ threads were coarser and branched more. The tips of these threads broke into free segments which surrounded themselves with a wall, assumed an olive-brown color, and germinated under favorable conditions.

### VITALITY OF SPORES OF USTILAGO TRITICI

An attempt was made to determine as far as possible the relative vitality of spores of the different species. Spores of *Ustilago tritici* col-

lected July 8, 1909, were kept in a dry place until December 10, 1909. Then some smutted heads were buried to the depth of about an inch in soil in pots and placed outside where they were fully exposed to the weather. On April 8, 1910, some of the spores were taken from the pots and placed in hanging drops in Ward cells beside other cells containing spores which had been in a dry place all winter. It was found that those spores which had been kept outside germinated as readily and the promycelia grew as vigorously as did those of the spores which had been kept in a dry place during the winter. Plate VII shows drawings of spores which had remained outside all winter and gives some idea of the character and vigor of germination.

## LOOSE SMUT OF BARLEY

*Ustilago nuda* (Jens.) Kell. and Sw.

### HISTORY

The earlier history of this smut is identical with that of *Ustilago hordei,* since the two forms were long considered the same. Lobelius (1581, p. 36) applied two names, viz., *Ustilago polystichi* and *Ustilago hordei distychi.* However, it is uncertain whether or not he separated loose barley smut from covered smut and put them under different names. Bauhin (1623, pp. 23, 24) applied the name *Ustilago hordei* to the smut on barley. It is not known whether or not he recognized the two forms. Persoon (1801, p. 224) gave the first really accurate description of barley smut, but he probably had in mind the covered and not the naked smut, since in his description he speaks of the spore mass as "pulvere latente."

It was not until 1888 that the loose and covered smuts were definitely separated. Jensen (1888b, pp. 405, 406) noticed the difference in the character of the spore-mass and the difference in color and size of the individual spores. He first considered what is now *Ustilago nuda* as a variety, giving it the name *Ustilago segetum* var. *hordei nuda.* Brefeld (1888, pp. 1592, 1593) concluded that the smut of barley and wheat was not the same as that on oats. He also studied the germination and noted the long, narrow promycelia producing no sporidia, as contrasted with the abundant production of sporidia in the oat smut and covered barley smut. He also asserted that the vitality of the spores was very low when compared with that of the spores of oat smut. Kellerman and Swingle (1890, pp. 277-285) worked out the details of germination carefully and gave the form specific rank. Brefeld, as late as 1895, insisted that the separation of loose smuts of barley and wheat had no sufficient basis.

The following is a synonymy of the species:

Ustilago. Bock, De stirpium, Historia Commentarius: 666. 1552.

Ustilago polystichi. Lobelius, Icones: 36. 1581. (According to Bauhin; see below.)

Ustilago hordei. Bauhin, Pinax Theatri Botanici: 23. 1623.

Reticularia Ustilago. Linné, Systema Naturae **2**$^2$:1472. 1791. (ed. 13.)

Reticularia segetum. Bulliard, Histoire des Champignons: 90. 1791. (According to Kellerman and Swingle, Ann. Rep. Kan. Agr. Exp. Sta. **2**:278. 1890.)

Caeoma D. Ustilago. Link, Observationes in Ordines plantarum naturales I. Magazin der Gesellschaft der Naturforschenden Freunde zu Berlin 3:5. 1809.

Uredo carbo a. Hordei. De Candolle, Flore française **5**:76. 1815. (Vol. 5 also called Vol. 6.)

Ustilago segetum. Ditmar in Sturm, Deutchlands Flora III. **1**:67. 1817.

Caeoma segetum. Link in Linné, Species Plantarum **6**$^2$:1. 1825. (ed. Willd.)

Erysibe vera a. Hordei. Wallroth, Flora cryptogamica Germaniae II. **4**:217. 1833.

Uredo carbo Hordei. Philippar, Traité sur la carie: 92. 1837.

Ustilago Carbo a. vulgaris c. Hordeacea. L. R. and Ch. Tulasne, Mémoire sur les Ustilaginées comparées aux Uredinées. Ann. Sci. Nat. Bot. III. **7**:80. 1847.

Ustilago segetum (Pers.) Ditt. b. Hordei. Rabenhorst, Klotzschii, Herbarium vivum mycologicum sistens fungorum: *397*. 1857. (ed. nov.)

Ustilago segetum var. Hordei f. nuda. Jensen, Om Kornsorternes Brand: 61. 1888. (a)

Ustilago Hordei. Brefeld, Neue Untersuchungen über die Brandpilze und Brandkrankheiten II. in Nachrichten aus dem Klub der Landwirthe zu Berlin 221:1593. 28 Je. 1888.

Ustilago segetum, var. hordei nuda. Jensen, Propagation and Prevention of Smut in Oats and Barley, Jour. Roy. Agr. Soc. Eng. **24**$^2$:407. 1888. (b)

Ustilago segetum, var. nuda. Plowright, British Ustilagineae and Uredineae: 274. 1889.

Ustilago Hordei nuda. Jensen, Le Charbon des Cereales: 4. 1889. (According to Clinton, N. A. Flora 7$^1$:8. 1906.)

Ustilago Hordei. Rostrup, Overs. K. Danske Vid. Selsk. Forh. **1890**:10. 1890.

Ustilago nuda hordei. Jensen, in letter to Kellerman and Swingle, dated January 24, 1890. (According to Kellerman and Swingle: 269. See below.)

Ustilago nuda. (Jensen) Kellerman and Swingle, Ann. Rep. Kan. Agr. Exp. Sta. **2**:277. 1890.

Ustilagidium Hordei. Herzberg, in Zopf, Beitr. Phys. Morph. Org. 5:7. 1895.

### DESCRIPTION OF SPORES OF USTILAGO NUDA

The spores of *Ustilago nuda* resemble quite closely those of *U. tritici*. In shape, size, color, and character of wall, the two are very similar, and it is sometimes difficult to distinguish between them. The spores are globose, subglobose, or oval, sometimes irregular in shape.

In color they are usually olive-brown or golden, but quite frequently they are of a darker, duller brown. They are very much lighter on one side than on the other. On the lighter-colored side are rather irregularly disposed, warty excrescences, except for a spot from which the promycelium later emerges. The spore wall seems thicker on the dark than on the light side. In comparison with *U. tritici* it seems thicker all around, and in most cases darker. Often the spores appear to be hollowed out toward the center, where they are also lighter in color than around the edge, thus giving the spore a concave appearance in optical section. The spore wall, as in *U. tritici,* consists of two parts, the epispore and endospore. Sometimes it is hard to distinguish clearly the two coats, but by the addition of potassium hydroxide they can be quite clearly seen. In size the spores vary quite considerably, averaging from 5 to 7 by 6 to 6½ μ, although some are larger, and the more elongate ones not infrequently are 10 or even 11 μ long. They may be distinguished sometimes from the spores of *Ustilago tritici* by the greater thickness of the spore wall and the somewhat concave appearance in optical section. However, this is not a universal characteristic; so it is sometimes almost impossible to distinguish between the two.

### GERMINATION OF SPORES OF USTILAGO NUDA IN WATER

The spores germinate quite readily in water, in shorter time than those of *U. tritici;* but they require a longer time than those of *U. hordei.* At room temperature about 25 per cent of the fresh spores had sent out promycelia within 12 hours.

The promycelium appears at first as a delicate, hyaline protuberance. As far as can be seen, it always arises from some point in or very near the lighter-colored area of the spore. The promycelium is usually not so much constricted at its base as is that of *U. tritici.* In fact it is very often constricted practically not at all (Plate VIII, *j*). Usually the spore wall is not ruptured by the promycelial tube, but in some cases it is slightly split (Plate VIII, *h* and *i*). This is especially likely to occur when the promycelia are short and blunt, in which case they seem to make up in thickness what they lack in length. The germ pore, then, must be large; it can not always be seen, but in the earlier stages of germination it is sometimes possible to see it quite distinctly.

At first there are no septa, but within the course of a few hours they appear. Eventually, in a full-grown promycelium, there are two or three septa, but some never become septate at all, while others have more than three septa. Three, however, is very commonly the number in a normal promycelium. Usually there are not many branches, but

sometimes they grow out near the septa (Plate VIII, *g*). Typically, then, the promycelium is rather long and slender, measuring about 25 to 50 by 2½ to 3 μ. Plate VIII, *l* shows a very characteristic form. Some fusions, mostly of the "knee-joint" type, were seen, but aside from these they were quite rare. So far as was observed, the promycelium remained attached to the spore throughout. Quite frequently segments became vacant, often the entire protoplasmic contents were lost, but even then the filaments did not separate. After 30 hours there were few changes in the original promycelium, except that some of the segments became vacant.

When the promycelium has reached its maximum size, very often a long, very slender germ tube is sent out from one of the "knee-joint" fusions, or from near a septum, or even from the tip of the promycelium. At first these germ threads are filled throughout their entire length with protoplasm, and may or may not be septate. In the earlier stages there are usually no septa, but in about 50 hours they begin to appear, especially as the thread grows, leaving the basal portion vacant. These germ threads keep on growing for 5 or 6 days, sometimes reaching a great length, from 299 to 450 μ. After a few days the basal portion is always vacant and septate, while the tip contains protoplasm and is usually non-septate. The basal portion is finally much longer than the tip. In a thread whose total length is 350 μ, the tip is ordinarily from 60 to 100 μ long. In diameter, the tips are about 2 μ wide, while the vacant, basal portion is usually narrower. The tips are very often wavy in outline, appearing much as did the swollen tips of *Ustilago tritici* before breaking up into resting cells. However, in *U. nuda* none of these tips were seen to break up, although the cultures were kept fully as long as those of *U. tritici*.

The germination in water is characterized by the development of a slender promycelium from a large opening in the spore; by the comparatively rare branching; by the development of long, very slender germ threads, and by the absence of free segments or sporidia.

### GERMINATION OF SPORES OF USTILAGO NUDA IN SUGAR SOLUTION

The early stages of germination in sugar solution are quite similar to those in water. The promycelia, however, are often much thicker, and, in consequence, the spore wall more often splits when germination begins. Branching is much more common and begins earlier. Often there are fair-sized branches in 20 hours, and in 27 hours they are sometimes quite extensive. The branches usually arise from the upper end of a cell, just below a septum, and fusions are commoner than in water. They may be "knee-joints," or they may be formed by

a branch growing out and fusing with the promycelium. Very often the intervening cells become vacant.

In 25 hours germ tubes begin to form. These grow out from the promycelium as lateral branches, as direct continuations of it, or from fusions. In 27 hours many of the promycelia had become wholly or partly vacant, and a simple or complex germ tube was growing out. Germ tubes also arise from branches, so that there may be a number of them from a single original promycelium. When these germ tubes grow out from fused portions, the fused parts themselves usually become vacant. The threads, as in other cases, become septate at the base, and the tip may branch, the branches in turn fusing (Plate IX, *a* and *b*). The basal portion of the germ tube does not necessarily become vacant first, although usually it does. An intermediate or even an end cell may become vacant while the others are still filled with protoplasm. Rarely two promycelia come from the same spore and each sends out a long infection thread; or sometimes the promycelium forks very near the base and two germ threads nearly equal in length arise in this way.

After 7 or 8 days a rather odd network, sometimes difficult to follow, is formed as a result of the growth and branching of a single promycelium (Plate 10, *b*, 2). In this particular case the promycelium sent out two branches very near the base. One of the branches sent out a long germ tube, while the other, after growing for a time, twisted back and fused with the original promycelium. From this fusion two germ tubes were sent out. The original branch also sent out a secondary branch, which in turn sent out a long, typical germ tube. Later a second promycelial filament (2) was sent out from the spore. This filament eventually fused at *b* with a protuberance of the branch which fused with the original promycelium. Later a germ thread was sent out from this fusion also, so that from the various branches and fusions of one original promycelium, five distinct, very long germ threads were sent out. Another specimen, showing the same general method of branching, fusing, and producing germ tubes, is shown in Plate XI, *b*. This is rather peculiar from the fact that at 16 days some of the promycelial segments still retained their protoplasm, although they had usually lost it long before that time.

The two cases just described are not oddities by any means. In fact such combinations as are shown in these figures occur quite frequently, but they could scarcely be considered as typical. More frequently the development is simpler and a single tube is produced from the promycelium, or two may be sent out which reach great length. After about 16 days the basal portion, which is almost hyaline,

is very hard to trace, so that some of the longest of the germ threads were probably not measured. A fairly representative one, picked out at random, measured 484 μ Some of them were longer and a length of 500 μ or more was not at all exceptional. The length seems all the more remarkable in view of the fact that the threads are very narrow, usually from about 2½ to 3½ μ wide. The tips are from 50 to 110 μ long, and usually wavy in outline; this wavy outline seems characteristic of the threads of *U. nuda,* since it did not appear so uniformly in any other form.

The tips keep on increasing in thickness for about 26 or 27 days, when some of them are 7 or 8 μ wide. Some are continuous and non-septate, while others at this stage seem to exhibit a tendency to constrict into rounded segments, as did those of *U. tritici* (Plate XII, *a*). From these rounded portions branches began to be sent out about the twenty-sixth day. Usually they were short, measuring from 25 to 35 μ, but in some instances they reached a considerable length (Plate XII, *a*). Sometimes these branches appeared very similar to the original germ tubes, the basal portion becoming vacant and segmented, and the tip still remaining filled with protoplasm (Plate XII, *b*).

Fusions between different filaments are quite common. Two of the protoplasmic tips, lying side by side, often send out short branches which fuse. Some filaments were observed in which two fusions took place (Plate XII, *g*); while cases in which branches of the same tube fused with each other, or a branch fused with the tip, were common. The culture in which the forms described above grew was kept for 46 days, but in that time it failed to show further important changes. It was kept in the expectation that resting cells such as were observed in *U. tritici* might be formed, but none appeared. In fact there were no free segments up to the forty-sixth day, unless the basal portion of the germ threads separated from the spores. This was not seen to occur, but it might easily have escaped notice, since after 35 days the filaments were hard to trace.

### SUMMARY OF GERMINATION OF SPORES OF USTILAGO NUDA

The characteristic features in the germination of this form are the development of a rather narrow, commonly 3-septate promycelium, which sends out very narrow infection or germ threads to great length, and the absence of sporidia, and non-liberation of free segments.

### VITALITY OF SPORES OF USTILAGO NUDA

Spores which were collected July 8, 1909, germinated very readily. Tests were made at intervals of about three weeks during the interim

between July and April of the next year. Not much difficulty was experienced at any time in obtaining a fairly large percentage of germinations. Some smutted heads were buried in pots December 12, 1909, and placed out of doors, where they were left until March 8, 1910, when they were tested for germination. These spores germinated normally, showing great vigor in their growth, especially in nutrient solutions. Plate XIII illustrates spores which germinated after having been outside all winter.

## COVERED SMUT OF BARLEY

*Ustilago hordei* (Pers.) Kell. and Sw.

### HISTORY

The history of this smut is rather uncertain. It was until so recently considered as identical with *Ustilago nuda* that there is question as to its exact history. The history of *U. nuda* has already been given, and the history of *U. hordei,* during the earlier period, is practically the same. Persoon (1801, p. 224) probably had the covered smut since he speaks of it as "pulvere latente," but he did not contrast it with loose smut of barley.

In 1888 Jensen (1888b, p. 406) definitely established the identity of the two smuts on barley. He then named the covered smut *Ustilago segetum* var. *tecta,* and in 1889 he called it *Ustilago hordei* var. *tecta.* Subsequently he considered this smut an independent species, giving it the name *Ustilago tecta hordei* Jensen. Kellerman and Swingle (1890, pp. 268-277), working out the spore characters and germination peculiarities of the various smuts, contributed still more to the knowledge concerning the species, and it was finally named *Ustilago hordei* (Persoon) Kellerman and Swingle. The synonymy of the species up to 1888, as far as can be determined, is identical with that of *U. nuda.* The synonyms subsequent to that time are given below. However, see reference to Persoon above.

Ustilago segetum var. Hordei f. tecta. Jensen, Om Kornsorternes Brand: 61. 1888.

Ustilago segetum var. hordei tecta. Jensen, Propagation and Prevention of Smut. Jour. Roy. Agr. Soc. Eng. **24**$^2$:407. 1888. (b)

Ustilago hordei v. tecta. Jensen, Le Charbon des Cereales: 4. 1889. (According to Kellerman and Swingle, see below.)

Ustilago tecta hordei. Jensen, in letter to Kellerman and Swingle. (According to Kellerman and Swingle, see below.)

Ustilago Jensenii. Rostrup, Overs. K. Danske Vid. Selsk. Forh. **1890**:12. 1890.

Ustilago Hordei (Pers.) Kellerman and Swingle. Kellerman and Swingle, Ann. Rep. Kan. Agr. Exp. Sta. **2**:268. 1890.

## DESCRIPTION OF SPORES OF USTILAGO HORDEI

The spore-mass of *Ustilago hordei* is different from that of either *U. tritici* or *U. nuda.* The spore-mass of the last two forms is dry and powdery, while that of *U. hordei* is usually more or less closely compacted, and seems sooty rather than powdery. In color the spore-masses of *U. nuda* and *U. tritici* are brownish black, while that of *U. hordei* is purplish or jet black.

In microscopic characters the spores are also different in important respects, those of *U. hordei* being, on the average, larger and rounder. More conspicuous is the fact that the spores in this form are absolutely smooth, there being no spines on the epispore. The spores vary somewhat in shape, but not as much as those of the two forms described, and, in a great majority of cases, they are subglobose or globose. They may be somewhat angled, but scarcely ever do they present the oval forms which are quite common in the two preceding species; the size is fairly uniform, the majority probably being between 6 and 9½ μ, although some are as large as 11½ μ in diameter. In color they are usually brown, and lack the bright luster which is often characteristic of *U. tritici* or *U. nuda,* being of a duller brown. There is sometimes a somewhat peculiar mottled appearance, due to the alternation of light and dark areas. The spores, as in the preceding species, are conspicuously lighter in color on one side than on the other, and on the dark side the spore wall often seems thicker.

## GERMINATION OF SPORES OF USTILAGO HORDEI IN WATER

The spores germinate very readily either in water or in nutrient solution, and germination proceeds in a much shorter time than in any of the other forms studied. A large percentage of fresh spores had germinated in 6½ hours, and nearly all germinated in 12 hours. As in the other forms, the promycelium grows out from some part of the lighter-colored area of the spore. The promycelial tube is often slightly curved, but may be straight. Septa appear in the course of a few hours and, when fully developed, the promycelium is usually 3-septate, although it may be more or less.

At about 15 to 18 hours, the promycelium has begun to branch and abjoint sporidia. A few vacuoles also appear at about this time. At 18½ hours there were already some vacant cells, but this was rare (Plate XIV, *c* to *e*). There seems to be no very definite order in which the cells lose their protoplasm. Any cell may first become vacant, and

the others follow in irregular order. At this time sporidia have already been formed; they may be abjointed either from the tip of the end cell or from septa, in which case they are usually pinched off just below a septum. In the formation of sporidia, a small protuberance is first sent out; this elongates, leaving a slender, very short basal portion by which it is attached to the promycelium, and then abstricts. Often it is possible to see the scar on the cell from which the sporidium was abjointed (Plate XIV, *h*). Usually the sporidia fall off almost immediately after formation, but more rarely they remain attached while they in turn bud and produce secondary sporidia. After one sporidium has fallen off from the promycelium, others may be produced in the same place, so that a continuous process goes on. Those already separated from the parent cell may grow and continue to form secondary sporidia by budding, but this is not so common in water as in nutrient solutions. In shape the sporidia are usually elliptical or broadly oval, and in size they average about 4 to 5 by 2 μ. Sometimes they germinate very soon after formation, but more often a considerable time elapses before they send out germ tubes.

At 48 hours a few germ threads had been sent out from promycelial cells. They arose in much the same manner as did those already described for the two preceding forms. In 48 hours they were already fairly well grown, the vacant, septate, basal portion averaging about 70 by 1½ to 2 μ, with swollen tips 20 to 30 μ long and a little wider than the basal portion. Vacant cells in the promycelium were now quite common, especially where branches or germ tubes were being sent out. In such cells the protoplasm disappears at first from the inner portion, leaving a film of protoplasm around the edge, but this may eventually disappear also.

After a few days practically all growth ceased, leaving such structures as have been described. A few fusions took place, but they were infrequent in occurrence. The tips of germ tubes swelled slightly, and a few of the sporidia germinated, but nearly always the tube sent out by the sporidium was short and not at all vigorous.

### SUMMARY OF GERMINATION OF SPORES OF USTILAGO HORDEI IN WATER

The spores germinate quickly, sending out from the lighter-colored portion of the spore a promycelium averaging about 45 μ in length. Sporidia are rather sparingly produced from tip and septa and may send out infection threads, but they are usually comparatively short. Branching takes place, but not to any such extent as in nutrient solutions.

GERMINATION OF SPORES OF USTILAGO HORDEI IN NUTRIENT SOLUTIONS

Very early there are marked differences between germination in water and in nutrient solutions; germination and growth in a nutrient solution being much more vigorous, some of the spores sending out two or in rare cases even three promycelia. When this occurs, there is usually more than one light-colored area in the spore, and it is from these that the tubes are sent out (Plate XV, *h*). The promycelia are uniformly thicker, although sometimes shorter, than those developed in water; often they are straighter, but they may be curved. The cells are shorter, thicker, and more constricted at the septa, giving them a plump appearance. Oil globules are more abundant than in water, both in the promycelia themselves and in the sporidia. The promycelium grows to a length of from 30 to 43 μ, although some are longer, and is ordinarily completely formed in 30 hours.

One of the most striking features appears in the vigor of growth, especially in the production of sporidia. Sporidia are abjointed in great profusion, both from the tips of end-cells and at the septa. Often they fall off very shortly after formation, but they may remain attached and go on budding very vigorously, producing secondary, tertiary, and even quaternary sporidia (Plate XV, *i*). After detachment the sporidia also may grow and keep on budding energetically, often from both ends, until, after about 30 or 40 hours, the culture is completely filled with them and even with the naked eye one can see on the cover glass a white mass of luxuriantly growing sporidia and filaments.

The sporidia vary in size and shape. They may be oval, elliptical, subglobose, or, rarely, somewhat elongate and slightly angular. In size there is also considerable diversity; when first abjointed they are 7 by 4 to 6 μ, but they later grow larger. They are thus seen to be larger than those produced in water. There is more of a tendency to assume a subglobose or globose form, the thickness being usually greater. The secondary sporidia may be of nearly the same size as those from which they are formed; usually they are a little smaller. For a few days the sporidia do not germinate, but keep on budding actively. After three days, however, some had sent out tubes to a length of from 20 to 25 μ. The tips of these germ tubes, even when short, are frequently swollen. Sporidia are sometimes formed from the tubes before they grow out into a long, typical germ thread.

As time passes on, the sporidia increase in size, as do the promycelia. Fusions between the branches of the same promycelia or different ones are quite common; and from these, or from cells of the

promycelium, germ tubes are sent out (Plate XVI). They resemble in general features of development and appearance those found in *U. tritici* and *U. nuda,* but are usually quite a little shorter and thicker. At about this stage many of the promycelia became detached and kept right on budding or branching. A few of the spores which germinated tardily sent out short, very thick, blunt, 3- or 4-septate promycelia, from which germ threads arose. Often from these spores there were two promycelia, one usually larger than the other (Plate XVI, *i* and *j*).

Upon the approaching exhaustion of the nutrient material, the tips of the germ tubes become swollen to about 6 or 7 μ and constrict, as do the short promycelial cells referred to above, into thick, very coarsely granular segments. Fusions between various segments, either of the same or of different filaments, are quite common. These segments may break apart when the nutrient material is exhausted; or they may remain attached. When they break up, they may separate into single segments, or a number may remain attached, forming a chain, as is shown in Plate XVII, *p*. The sporidia have by this time grown to a considerable size, many of them being as large as the original spore. The culture was in this condition at 9 days, when the cell was allowed to dry. More sugar solution was then introduced and within 10 hours the segments which had formed sent out tubes (Plate XVII, *d, g, m, p, q, r, s*). These tubes often resembled original promycelia, while in other cases they were much like germ tubes (Plate XVII, *d*). The sporidia also send out germ tubes or keep on budding. Sometimes the sporidia germinate from one end and keep on budding from the other. The growth in the culture became very rank by the end of 12 days; in fact there was such a conglomeration of budding and germinating sporidia, free segments, germ tubes, and promycelial remains that accurate observation was made very difficult. In the dense masses, however, sporidia did not germinate; neither did free segments, but only those around the edge sent out tubes.

### SUMMARY OF GERMINATION OF SPORES OF USTILAGO HORDEI IN SUGAR SOLUTION

The essential features of the germination in sugar are the abundant production of sporidia which may keep on producing by budding other sporidia, or may germinate, sending out long germ threads. The frequent occurrence of free segments, and their long continued activity, and the typical formation of resting segments upon approach of unfavorable conditions, are also characteristic. The resting segments formed earlier in this form than in *U. tritici* and germinated

more readily. This seems very natural in view of the fact that there is a greater tendency towards formation of free segments and a more ready germination in *U. hordei* than in *U. tritici.*

### VITALITY OF SPORES OF USTILAGO HORDEI

The ability of spores successfully to undergo winter conditions was tested in the same way as in the two other forms so far described. There was no apparent diminution of vitality or vigor due to the exposure during the time the spores were outside.

## SMUT OF OATS

*Ustilago avenae* (Pers.) Jens.

### HISTORY

The ancients probably knew the smut of oats, but did not distinguish it from other smuts. For a long time it was not recognized as a fungus parasite, but considered as an abnormality of the plant itself. Linnaeus at first failed to recognize it as a fungus, but later he did so. Bock (1552, p. 666) gave it the name *Ustilago,* while Lobelius (1581, p. 36) and C. Bauhin (1623, p. 24) called it *Ustilago avenae* and *Ustilago avenacea* respectively. None of these men recognized the true nature of the malady; in fact, some even considered that it was due to the presence of small animal organisms.

Bulliard (1791, p. 90) recognized it as a fungus, but did not distinguish between the different smuts. He applied the name *Reticularia segetum.* A number of writers between the years 1750 and 1800 mentioned the loose smuts, but few of them took any particular notice of the smut on oats.

Persoon (1801, p. 224) adopted Bulliard's specific name for the loose smuts in general, but he separated them into varieties on the different hosts, calling the one on oats *Uredo* (*Ustilago*) *segetum* g. *Uredo avenae.* De Candolle (1815, p. 76) called all the loose smuts *Uredo carbo,* retaining for the forms on various hosts the varietal names of Persoon. The oat smut thus became in his system of nomenclature, *Uredo carbo* g. *avenae.* Other names were applied but not a great deal of real knowledge was added until Jensen made his inoculation tests and showed the various forms distinct. His ideas were substantiated by the extensive germination tests made by Brefeld and by Kellerman and Swingle, to which attention has been called in the general historical introduction. Oat smut, together with other "loose" smuts, was referred to as *Ustilago segetum* (Bull.) Ditm., and as

*Ustilago carbo* (D. C.) Tul. Jensen, however, removed the necessity for confusion so far as oat smut is concerned by naming it first *Ustilago segetum* var. *avenae* in 1888 (1888a, p. 61), and *Ustilago avenae* in 1889 (1889, p. 4), basing his conclusions on cross-inoculation experiments.

The following is a synonymy of the species:

Ustilago Avenacea. Lobelius, Stirpium Icones: 36. 1581. (According to Bauhin, see below.)

Ustilago Avenae. Bauhin. Pinax Theatri Botanici: 24. 1623.

Reticularia Ustilago. Linné, Systema Naturae **2**$^2$:1472. 1791. (Ed. 13.)

Reticularia segetum. Bulliard, Histoire des Champignons **1**:90. 1797. (According to Persoon, see below.)

Uredo segetum c. Avenae. Persoon, Tentamen dispositiones methodicae fungorum: 57. 1797.

Uredo (Ustilago) segetum g. Avenae. Persoon, Synopsis methodica fungorum **1**:224. 1801.

Caeoma D. Ustilago. Link, Observationes in Ordines plantarum naturales I. Magazin für die Neuesten Entdeckungen in der gesammten Naturkunde der Gesellschaft Naturforschender Freunde zu Berlin **3**:6. 1809.

Uredo carbo c. Avenae. De Candolle, Flore française **5**:76. 1815. (Vol. 5 also called Vol. 6.)

Ustilago segetum. Ditmar, in Sturm, Deutschlands Flora III. **1**:67. 1817.

Caeoma segetum. Link, in Linné, Species Plantarum **6**$^2$:1. 1825. (ed. Willd.)

Erysibe vera c. Avenae. Wallroth, Flora cryptogamica Germaniae II. **4**:217. 1833.

Uredo carbo-Avenae. Philippar, Traité sur la carie, le Charbon, etc.: 91. 1837.

Ustilago Carbo a. vulgaris b. Avenacea. L. R. and Ch. Tulasne, Mémoire sur les Ustilaginées comparées aux Uredinées. Ann. Sci. Nat. Bot. III. **7**:80. 1847.

Ustilago segetum, var. avenae. Jensen, Propagation and Prevention of Smut in Oats and Barley. Jour. Roy. Agr. Soc. Eng. **24**$^2$:407. 1888.

Ustilago Avenae (Pers.) Jensen, Le Charbon des Cereales: 4. 1889. (According to Clinton, N. A., Flora. **7**$^1$:7. 1906.)

Ustilago avenae f. foliicola. Almeida, Revista agronomica **1**:22. 1903.

## DESCRIPTION OF SPORES OF USTILAGO AVENAE

The spores resemble somewhat those of *Ustilago nuda* and *Ustilago tritici*. In size they are very nearly the same, ranging from 5 to 11 μ. They lack, however, the luster of the spores of the two forms just mentioned. Whereas those of *U. tritici* and *U. nuda* often have a golden tinge, those of *U. avenae* are usually of a dull brownish color, except in the lighter-colored areas. In shape they approach more often subglobose or globose than those of *U. tritici*. Sometimes they are oval or elliptical, but not typically. They may sometimes be angular and somewhat irregular in outline. As in the cases of the other

spores mentioned, they are conspicuously lighter in color on one side than they are on the other. On the light side the echinulate character of the spore wall is very often clearly perceptible. On the darker side it is possible more often than in other smuts to see the endospore without the use of reagents. When visible, it usually appears as a faintly outlined hyaline portion just inside of the epispore.

### GERMINATION OF SPORES OF USTILAGO AVENAE IN WATER

The spores germinate quite readily in from 10 to 13 hours. As in the other forms, the promycelium extrudes from the lighter-colored side of the spore. Two germ tubes are not uncommon; when there are two, both usually grow out from the lighter-colored side of the spore (Plate XVIII, *h*). The promycelia are at first rather slender and non-septate. Very shortly, however, septa begin to appear. In 24 hours the promycelia are often fully grown, measuring from 20 to 40 μ long by 4 to 5 μ wide. They are either straight or slightly curved, and usually 2- or sometimes 3-septate. They abjoint sporidia from the septa or from the tip, more often from the tip, although it is not at all uncommon for them to be formed at the septa. They are pointed at the end by which they were attached, are oval or elliptical, sometimes elongate in form, and measure 4 by 2 to 7 by 7 μ. These sporidia may again bud, either *in situ* or after detachment, and form secondary spores. This, however, was not very prevalent in water, occurring by no means as often as in nutrient solutions.

When fully grown the promycelia have from 1 to 3 or more septa but the usual number of septa is 2. Quite frequently branches arise from some point near the base of the promycelium, which in some cases grow up and fuse with one of the promycelial cells. A very prevalent tendency is the production of knoblike protuberances on opposite sides of a septum. These stubby branches fuse and there is formed as a result a knob at the septum. As the promycelia grow, the parts of the knob are forced apart, and such a condition as is shown in Plate XVIII, *g*, appears. Very often the promycelium is quite sharply bent at these fusions (Plate XVIII, *m*).

Usually after a few days the sporidia have germinated; in water few are formed after about 2 days. They may eventually send out a long infection thread, showing the typical characteristics of those already discussed for the other forms. The promycelia in the meantime often become detached and swell up, the swelling being especially apparent at the ends of the cells which seem rather loosely held together. The protoplasm becomes vacuolated, many of the cells becoming entirely vacant, while those which retain their protoplasm keep

on swelling slightly until they are often as thick as the original spore. Long germ threads are now usually sent out, and with their formation the culture usually becomes dormant.

### SUMMARY OF GERMINATION OF SPORES OF USTILAGO AVENAE IN WATER

The germination and growth in water is characterized by the meager production of sporidia, and by the fact that they seem to be formed from the tip of the promycelium more frequently than from the septum. The promycelia are somewhat shorter than those of other smuts and are usually 2-septate, although not necessarily so, and they very frequently become detached. Very often they form "knee-joint" fusions, and eventually either become vacant or swell up at the ends and send out germ tubes before becoming vacant. Infection threads are sent out either by sporidia or from one of the cells, or from a fusion tube of the promycelium.

### GERMINATION OF SPORES OF USTILAGO AVENAE IN NUTRIENT SOLUTION

In nutrient solutions growth is always much more vigorous than in water. Quite frequently two promycelia are sent out from the same spore. Often after the promycelium is fairly well grown, another one is sent out, or in some cases two are sent out simultaneously.

Sporidia are formed in much greater abundance than is the case in water (Plate IX, *f, g, l, p,* etc.). They are abjointed both from septa and from the tips of the end-cells. Ordinarily they fall off soon after formation, but they sometimes remain attached and produce secondary sporidia. Usually they are larger than those formed in water. As in the case of *U. hordei,* they are typically of a different shape. Those formed in water were elliptical or oval, while those formed in sugar solution are very often subglobose. In size they averaged, shortly after detachment, about 5 by 9 μ.

Promycelia often become detached and keep on budding just as though they were still attached. The sporidia also keep on budding very vigorously after detachment (Plate XIX, *o*). When the nourishment begins to be exhausted, however, this activity seems to cease and germ tubes are sent out. The drying of the culture does not seem to impair the vital properties of either the thickened promycelial cells or the sporidia, for upon the addition of more nutrient solution they again become active.

The chief difference between germination in water and in nutrient solution is the greater vigor of germination in sugar solution and the

more abundant and prolonged production of sporidia. In other respects, such as branching, fusions, production of germ tubes, and freeing of segments, there is no vital difference. The vitality of the spores is not lessened by exposure to winter conditions and germination is nearly as prompt and vigorous as when the spores are fresh.

## SMUT OF CORN

*Ustilago zeae* (Beckm.) Unger

### HISTORY

Aymen (1760, p. 77), in about 1750, seems to have been the first to take much notice of maize smut. He thought that the source of the disease was in the tassels. De Candolle (1805, p. 596) included it under the name *Uredo segetum,* designating it as a variety, *mays zeae.* Unger (1833, p. 350) discussed it somewhat, making observations on both the gross and minute structure. Léveillé (1839, p. 9) gave an incomplete description of microscopic characters, and Tulasne (1847, p. 83) gave a description of the smut, together with a synonomy of the species. De Bary (1853, p. 4) gave a detailed account of the mycelium in the tissues of the host, and of the development of the spores. Kühn (1859, p. 70) tried to germinate the smut spores, but found that fresh spores could not be induced to germinate in water with any degree of readiness. He found, however, that in the following January they germinated fairly well. Brefeld (1883, p. 68) gives a complete and interesting account of their germination, his experience with the germination of fresh spores in water being similar to that of Kühn. He received a smut "boil" from a friend and at once attempted to germinate the spores in water, but was surprised to find his efforts not at all successful. Subsequent to this time he accidentally dropped the spore-mass, and a cloud of spores arose, some of them falling into cultures of other spores growing in nutrient media. He found that these spores germinated very readily. However, he was not successful in obtaining germinations in water until the following spring. He describes in detail the various phases of germination, and later gives the results of extensive infection experiments which he carried on. Hitchcock and Norton (1896) gave a complete history of the smut, together with original results on the life-history and spore germinations.

The following is a synonymy of the species:

Lycoperdon zeae. Beckmann, Hannoverisches Magazin 6:1330. 1768.
Uredo segetum d. Mays zeae. De Candolle, Flore française 2:596. 1805.
Uredo zea mays. Lamarck, Encyclopedia methodique botanique 8:227. 1808.

Uredo maydis. De Candolle, Flore française 5:77. 1815. (Vol. 5 also called Vol. 6.)

Uredo (D. Ustilago) Zeae. Schweinitz, Synopsis fungorum Carolinae superioris: 71. 1822.

Caeoma Zeae. Link, in Linné, Species Plantarum $6^2$:2. 1825. (ed. Willd.)

Erysibe Maydis. Wallroth, Flora cryptogamica Germaniae II. 4:215. 1833.

Ustilago zeae (Beckm.). Unger, Einfluss des Bodens, u. s. w.: 211. 1836.

Uredo carbo maiadis. Philippar, Traité sur la carie: 93. 1837.

Ustilago Maydis. Corda, Icones fungorum 5:3. 1842.

Ustilago Schweinitzii. Tulasne, Ann. Sci. Nat. Bot. III. 7:86. 1847.

Ustilago Euchlaenae. Arcangeli, Erb. Critt. Ital. II. *1152*. (According to Clinton, North American, Ustilagineae. Proc. Boston Soc. Nat. Hist. **31**:362. 1904.)

Ustilago zeae Mays. (D C). Winter, Die Pilze, in Rabenhorst, Kryptogamen flora **1**:97. 1884. (ed. 2.)

Ustilago Mays zeae. Magnus, Deutsche botanische Monatsschrift **13**:50. 1895. (According to Clinton, North American Flora $7^1$:15. 1906.)

## DESCRIPTION OF SPORES OF USTILAGO ZEAE

In shape the spores are sometimes elliptical and infrequently irregular, but by far the greater percentage are either globose or subglobose. They are very prominently echinulate, the spines showing distinctly on all sides. Unlike all the other spores so far described, those of *Ustilago zeae* are not lighter on one side than on the other. In size they are larger than any of the four already described, varying from 8½ to 15 μ. They are usually about 10 μ in diameter and seem to vary less from the normal than do those of *Ustilago tritici* and *Ustilago nuda*. Very few were seen which measured 15 μ, although a few abnormally long ones did. When that long, however, they were considerably thinner than the average, being of a narrowly oval shape, which is rather uncommon.

They are usually reddish brown in color, although some are darker, especially around the edges in optical section, where they are often brownish black. The wall consists, as in other forms, of two parts, the outer, or epispore, and the inner, or endospore. The endospore is sometimes difficult to see without the aid of reagents. However, when treated with either potassium hydroxide or acetic acid, the two coats can be very clearly seen.

## GERMINATION OF SPORES OF USTILAGO ZEAE IN WATER

The spores collected at St. Anthony Park, Minnesota, especially when fresh, germinated either very tardily or not at all in water. Fresh spores, just taken from the plant, were tried, but they failed to germinate consistently. Many attempts were made during the fall

and early winter of 1909-1910 to germinate them, but with little success. It was not until early in January that they could be induced to germinate. Only once or twice were fresh spores observed to germinate. A small fraction of one per cent of fresh spores germinated in the fall of 1910. (Spores collected and tried in the summer of 1913 germinated very readily when fresh.) It required two days for them to begin germinating, in both water and sugar solution. Chains of "air-conidia" were formed in both.

The experience of Brefeld and Kühn has already been cited. As opposed to their experience and the results obtained here, Hitchcock and Norton (1896, p. 196) found no difficulty in germinating fresh spores in water; in fact it was their experience that fresh spores germinated more readily, although a little less luxuriantly, in water than in nutrient solutions.

The spores, after a resting period, germinate fairly well in water, requiring less than 14 hours. The promycelium, more often than in preceding forms, ruptures the spore wall. Ordinarily the rent in the wall is only short, but it is very often conspicuous. The promycelium is at first very delicate and non-septate. The base is often constricted where the promycelium emerges from the spore (Plate XX, *a* to *i*). Within 20 hours septa usually appear, and at about the same time there are outgrowths from near the septa. These delicate protuberances develop into small fusiform conidia, measuring from 8 to 18 by ½ to 2 μ. Usually the conidia break off very soon after formation, and only rarely were they observed to produce secondary conidia *in situ*. However, after being abjointed, they very often formed secondary conidia, usually smaller than themselves. Occasionally the promycelia partly lost their protoplasmic contents in the formation of sporidia (Plate XX, *h*), but this was not so often the case with attached as with detached promycelia.

When full grown, usually within 25 hours or less, the promycelia range in length from 20 to 60 μ and are comparatively narrow. They usually have 3 septa, but may have only 2. They may be either straight or curved, but usually when they are slender they are also curved. They may or may not be branched, and, when branches appear, they often arise from the base (Plate XX, *e*). The promycelia become detached very readily. At first either the entire promycelium or two of the cells break off, leaving the others attached to the spore. Later the remaining cells separate from the spore, so that in 4 days there are practically no attached promycelia to be found. The detached promycelia do not seem to suffer any diminution of vegetative activity in consequence of their detachment from the spore. They may

even keep on growing, and frequently do, or they may produce conidia just as vigorously as they did while still attached to the spore. Often they become vacuolated, and later lose their protoplasm entirely as a result of the continuous formation of sporidia (Plate XX, *o, p*). Sometimes short chains of conidia are formed in the water, but this chain formation is much more apt to occur when the promycelia of spores growing on the surface of the water in watch crystals are partially or wholly exposed to the air. In such cases chains of considerable length are often formed (Plate XX, *a*). This figure is of air-sporidia in a manure decoction, but they are very similar to those formed in watch crystals filled with water.

The promycelia, sometimes while still attached, and often after detachment, send out a germ thread, protoplasmic at first, but soon becoming vacuolated or entirely vacant, but segmented in the basal portion (Plate XX, *g*). The sporidia, too, germinate quite readily. Some had already germinated at the expiration of 24 hours, while others required a much longer time. At first the filament is narrow and often wavy in outline, but as growth progresses these infection threads assume practically the same character as those sent from the promycelial segments. The sporidia themselves become vacant after the germ tube is well established. One peculiarity is the total absence of fusions, none of any nature being seen. After 5 days very little, if any, change took place. The germ tubes may have grown a little, but it was almost imperceptible.

### SUMMARY OF GERMINATION OF SPORES OF USTILAGO ZEAE IN WATER

Fresh spores germinate with difficulty, or not at all, in water; but after a resting period they germinate quite readily.* The promycelia are rather slender, usually have 2 or 3 septa, and are often slightly curved. Rather small fusiform sporidia are sparingly produced, mainly from the septa. Rarely these may form other sporidia, but more frequently they send out infection threads. The promycelium usually separates from the spore, continuing its activities as though still attached. There were no fusions observed, which is in contrast with other smuts. The germ tubes from either promycelia or sporidia were in no case as long as those of other forms.

### GERMINATION OF SPORES OF USTILAGO ZEAE IN NUTRIENT SOLUTION

The promycelia produced in nutrient solution are usually much shorter and blunter than those produced in water (Plate XXI, *e′* and *e″*). The base is not so often constricted and in fact very often the base

* See, however, statement of results in 1913, on page 36.

is thicker than the portions out toward the tip, thus giving a pyramidal appearance. The promycelium, as in water, has 2, 3, or even 4 septa and abjoints sporidia from septa, or in some cases from the tip. The sporidia are much more numerous than in water and within a couple of days the culture teems with them. They may either bud again, forming secondary sporidia, or they may send out germ tubes soon after formation. More often they keep on budding while the nutriment lasts, sometimes forming masses of sporidia so dense that only those around the edge ever germinate. They are usually considerably larger than those formed in water, in some cases measuring as much as 30 by 5 μ. Their shape is also different; they have rounded edges and are thicker, being less often spindle-shaped. Their large size, together with the fact that they are often of the same shape as individual segments of the dismembered promycelium, makes it rather difficult at times to distinguish between them. Upon germination the sporidia give rise to long, slender germ threads, resembling quite closely those formed in water. The promycelium may branch and send out filaments such as are shown in Plate XXI, *c;* or it may break up, go on budding, and finally send out germ tubes from the various segments. When growing on the surface, long, much-branched chains of air-conidia are produced, differing from those in the liquid mainly in the fact that they do not break apart so readily as those in the solution. They are also sometimes thicker-walled and resemble ungerminated sporidia in having a large supply of oil. (See Plate XXII.)

#### SUMMARY OF GERMINATION OF SPORES OF USTILAGO ZEAE IN NUTRIENT SOLUTION

The promycelia formed in a nutrient solution are thicker and sometimes shorter than those formed in water. Sporidia are much more abundantly produced, are larger, more oval in shape, and bud more vigorously than in water (Plate XXII). Sporidia are produced as long as the nutrient material lasts; when it is exhausted, or approaches exhaustion, germ tubes are sent out either from sporidia or from promycelial segments. Spores germinate very readily after having been outdoors all winter.

## STINKING SMUT OF WHEAT

*Tilletia foetens* (B. and C.) Trel.

#### INTRODUCTORY

This is the smut popularly known as "bunt" or stinking smut of wheat. There are two species of bunt which can not very well be dis-

tinguished except by microscopic examination of the spores. *Tilletia foetens* (B. and C.) Trel., the form described here, has smooth spores, while *Tilletia tritici* (Bjerk) Winter, has very pronounced winged reticulations. In size they are nearly the same, although those of *Tilletia tritici* are a little smaller. Both of these forms occur in the United States. Both are also found in Minnesota, where this work was done, but *Tilletia foetens* is the more common form. All of the bunt collected at St. Anthony Park, Minnesota, was of the latter species, and specimens obtained from St. Peter, Belgrade, St. Hilaire, Detroit, Olivia, Watson, and Mendota, representative of different sections of Minnesota, also proved to be *Tilletia foetens.* The places in Minnesota from which spores were obtained are fairly representative of the different sections of the State, so it is probable that there is not a very distinct regional distribution of the two forms, but rather a local one. Out of a great many examinations of smut balls found in seed wheat from different parts of Minnesota, only one showed the presence of *Tilletia tritici* spores.

## HISTORY

The early history of *Tilletia foetens* is confused with that of *T. tritici.* The ancients knew the stinking smut of wheat, but there is, of course, no way of telling which species they described. It was long known as *Uredo caries,* having been thus referred to by De Candolle (1815, p. 78). Tulasne (1847, p. 113) designated stinking smut as *Tilletia caries.* He undoubtedly had in mind *Tilletia tritici,* since the spores are figured with reticulated thickenings.

The smooth-spored stinking smut was called *Ustilago foetens* at first (1882, p. 139), being thus described in 1860. Kühn (1873, No. 1697, p. 152), in Germany, designated it as *Tilletia laevis* and it is thus referred to by Frank (1896, p. 117) and other European writers. Kühn mentions the fact that the spores germinate in the same way as do those of *Tilletia caries,* but that the spores are constantly different, it being, therefore, not merely a variant form but a true species.

The germination of Tilletia forms was described by Prevost (1870, 1807
p. 281), De Candolle (1832, p. 1436), Tulasne (1854, pp. 161-163), Kühn (1859, p. 54), Fischer von Waldheim (1869, p. 64), Brefeld (1883, p. 146), and others. All noted the rather uncertain and capricious germination.

The following is a synonymy of the species:

Ustilago foetens (B. and C.). Ravenel, Fung. Car. Exsicc. *V. 100.* 1860. (According to Trelease, Preliminary List, Wisconsin Parasitic Fungi. Trans. Wis. Acad. Sci. **5**:139. 1882.)

Tilletia laevis Kühn. Rabenhorst, Fungi Europaie, Exsiccati 1697. 1873.

Tilletia foetens (B. and C.). Trelease, Preliminary List, Parasitic Fungi of Wisconsin. Trans. Wis. Acad. Sci. **5**:139. 1882.

### DESCRIPTION OF SPORES OF TILLETIA FOETENS

The spores are the largest by far of all those of the species studied. They are from 14 to 27 μ, usually about 20 μ in diameter. In shape they are usually globose, or sometimes subglobose, but occasionally more elongate and oval. The wall is composed of epispore and endospore. The epispore is entirely smooth like that of *Ustilago hordei*. The two spore coats are more easily distinguishable than those of the other smuts, probably because of the greater size of the spores. The addition of potassium hydroxide makes it possible to see them very clearly. The epispore is often dark brown, sometimes of a lighter color, while the endospore is almost hyaline. In color the spore is usually light or dark brown, sometimes almost golden. Some of the spores are of the same color throughout, presenting a bronze-colored surface, while others have a large number of oil globules, such as the spores shown in Plate XXI, *e*′ and *e*″.

### GERMINATION OF SPORES OF TILLETIA FOETENS IN WATER

Spores collected in August, 1909, were immediately tested for germination, but none germinated either in water or in nutrient solutions. Repeated attempts were made throughout the fall and winter, but always with negative results. Some smutted heads were put outside December 10, 1909, and allowed to remain there until April 8, 1910, when some were put in hanging drops in Ward cells. These spores germinated in 72 hours. Brefeld (1883, p. 147) states that the spores of *Tilletia tritici,* which he tried, germinated readily when fresh. Spores of *T. tritici* from Pullman, Washington, were tried repeatedly in the fall and winter, but did not germinate, while those which had been frozen germinated within 3 days. Fresh spores, however, germinated in the fall of 1910. Spores of *Tilletia foetens* and some of *Tilletia tritici* were collected July 21, 1910. Germination tests were made 5 days later. For these tests distilled water, 5 per cent sugar solution, and soil infusion, were used. Only those in distilled water germinated. The spores of *Tilletia foctens* began to germinate in 11 days, about 20 per cent of them eventually germinating, while those of *Tilletia tritici* required 17 days; about the same per cent sending out germ tubes. Spores kept in the light and in the dark germinated equally well. In tests made during the latter part of April, 1910, spores of *T. foetens* germinated in 48 hours. Not more than 10 per cent had germinated by this time, but in 70 hours practically every spore had

sent out a germination tube. The epispore is nearly always ruptured when the promycelium is formed. Sometimes the rent is not very long, extending not more than one sixth of the length of the spore but in other cases it is long and very conspicuous, often reaching very nearly the length of the spore. In many cases there was a very decided crack from which others extended (Plate XXIII). Sometimes part of the endospore seems to be carried out of the spore with the promycelium and then torn, especially when an unusually thick promycelium is sent out. The promycelium appears first as a straight or sometimes wavy protuberance, sometimes coarsely granular, but usually not exceptionally so (Plate XXIV, *a-s*). The spores which germinate late often send out much thicker and blunter promycelia, which frequently branch almost as soon as they extrude from the spore (Plate XXIV, *g* and *h*). The branches in these cases are, however, nearly always short and stubby, appearing at first like irregular knobs. They frequently have a gnarled appearance and are almost as broad as the spore itself. The more prevalent this tendency is the longer germination is delayed.

Normally the promycelia are very much like those shown in Plate XXIV, *f* and *p*. Septa are rare or absent in filaments that have no vacant portion. Within a short time vacuoles appear, however, and, as the promycelium grows, the protoplasm moving continually forward, the portion next to the spore becomes vacuolated, then entirely vacant, and finally septate. Sometimes a promycelium may lose its protoplasm without becoming septate (Plate XXIV, *n*). Simple or compound branching is very common at this stage and in most cases the branches are very nearly as large as the original promycelium. In 76 hours some of the promycelia assumed the characteristic appearance of germ threads, and in 96 hours practically all of them had taken on this form, but they grew for some time longer. They varied in length from 90 to 250 μ, thus being rather short in comparison with *U. tritici* and *U. nuda,* especially when the relative sizes of the spores are considered. The tubes continued to produce vacant cells until in 7 days many of them were entirely vacant and hyaline. In 8 days the filaments had nearly all disintegrated, or at least were dormant and ready to break up. This is an account of a germination in a Ward cell and many more like it were observed. No sporidia were formed nor was there any apparent tendency toward their production except in a few cases.

Spores were also put in water in watch crystals to determine whether or not there were important differences from those germinating in Ward cells. Many of these spores sent out promycelia in 48

hours. In 75 hours small protuberances began to appear on the tips of some of the promycelia which still retained all of their protoplasm. These knobs varied in number and as they were not all visible, it was sometimes difficult to determine the exact number with certainty. They grew out into finger-like projections and finally into an even number of long, slender, somewhat cylindrical or narrowly sickle-shaped sporidia. In 4 days these sporidia were very numerous.

Usually there were 4, 6, 8, or 10 sporidia, 6 and 8 being very common. Odd numbers sometimes occur, but only as rare exceptions. At first the sporidia are arranged in a whorl on the end of the promycelial tube. Often they diverge toward the distal extremity, but in some cases they converge, forming a spindle-shaped bundle. The individual sporidia are at first well filled with protoplasm, containing but few vacuoles, and as far as can be seen, no septa. Often, while still attached to the promycelium, little knoblike branches were sent out toward each other by neighboring sporidia. These branches meet and fuse. Often all the sporidia of one whorl are united in pairs. The sporidia may become detached from the promycelial tube before fusion; in that event, they fuse with the nearest sporidium, or, if none be in close proximity, they fail to fuse. However, the chances of one sporidium's becoming isolated are not very great, since they are produced so close together.

One of the pair of fused sporidia now often sends out a slender branch, commonly from near the tip, but not necessarily so (Plate XXV, *h* and *h'*). This branch grows out into a very long, narrow, often much-coiled germ tube, filled with protoplasm at first, but eventually becoming vacant and segmented from the base outward, and retaining protoplasm only in the somewhat swollen tip. As the germ tubes increase in length, the protoplasm of the sporidia becomes vacuolated at first and finally disappears altogether (Plates XXIV and XXV). At the same time septa, varying in number from 5 to 7, appear in the fused sporidia, which, now fully grown, are often from 70 to 80 μ long by 3 to 4 μ wide. It is not absolutely essential that a sporidium fuse in order that it may send out a germ tube, but in the great majority of cases the fusion takes place.

Instead of immediately sending out germ tubes, the sporidia often send out short, rather thick little branches, which gradually swell up toward the tip. These branches keep on getting larger toward the tip until they appear to be attached by a slender filament resembling a sterigma. These are secondary sporidia and are often bean-shaped or crescent-shaped, reaching from 20 to 45 μ in length, and from 4 to 6 μ in width. They may send out long slender germ tubes very closely

resembling those sent out by the primary sporidia, or, in some cases, they send out a filament which keeps on producing more secondary sporidia (Plate XXV, *d*). The germ threads may branch from the filled tip and may produce more secondary sporidia from these lateral branches (Plate XXV, *g*).

When mature, the germ threads are very long, some of them measuring at least 450 μ with a tip from 75 to 110 μ long. Some of them may very probably be longer, but on account of the fact that they have so strong a tendency to become much coiled, absolutely accurate measurements are very hard to make.

### SUMMARY OF GERMINATION OF SPORES OF TILLETIA FOETENS IN WATER

The spores seem to require a resting period; at any rate they germinate more readily after a period of rest. Germination occurred in from 2 to 4 days, depending upon conditions. The spore wall in most cases is ruptured by the simple or branched promycelium, which grew to a length of about 200 μ and assumed the appearance of a germ thread. In case the promycelium reaches the air, long, cylindrical, often curved sporidia are produced in whorls on the tip. These unite in pairs before or after detachment from the promycelium, and either send out long infection threads or produce secondary sporidia which send out the threads. On these threads, produced by secondary sporidia, short lateral branches may arise which in turn produce sporidia like the one from which the thread grew. Although usually the secondary sporidia produced by a germ tube are formed on lateral branches, sometimes they are formed in a string. In case there is an odd number of sporidia, or if fusion fails for any other reason to take place, the single sporidium may send out a germ tube, but it is shorter than the average.

### GERMINATION OF SPORES OF TILLETIA FOETENS IN NUTRIENT SOLUTION

In practically all trials in nutrient solutions, except soil infusion, the presence of the nutriment seemed to have a deleterious effect on both *Tilletia foetens* and *T. tritici*. The spores, under the same conditions of temperature, etc., required a longer time to germinate than they did in water. The germinations, when they did occur, were usually abnormal. In a large number of cases the promycelia exhibited a very well-defined and persistent tendency to form an irregular or sometimes even subglobose or globose mass of protoplasm, resembling somewhat an overgrown amoeba with few or no pseudopodia. Occa-

sionally the entire contents of the spore seemed to be making an unsuccessful effort, as it were, to come out of the spore at the same time. In rare cases even the endospore became ruptured and the whole pushed partly out of the epispore, like the contents of a grape partly squeezed out of the skin. In these cases no promycelium was sent out, although the distal portion sometimes became somewhat pointed, as if to send out a tube. However, there was seldom much growth from such protoplasmic masses. After from 7 to 9 days the whole became vacant, leaving a bubble-like remnant. When promycelia were sent out, they were short-lived, and in no case were they seen to produce sporidia.

## GENERAL SUMMARY

The smuts considered fall naturally, as far as germination is concerned, into two general classes: those which produce sporidia and those which produce no sporidia. In the second class are included *Ustilago nuda* and *Ustilago tritici;* while all the others belong to the first class. The first class may be subdivided into those producing sporidia mainly at the septa and those producing them in whorls at the end of the promycelium. In the former are included the following: *Ustilago zeae, U. avenae,* and *U. hordei,* while in the latter subclass is included only *Tilletia foetens.*

The period required for germination varies considerably in different forms. *U. hordei* germinates most readily, only 6½ hours being required in some cases. *Tilletia foetens* requires the longest time, the minimum for these spores being 48 hours. The intermediate forms show differences of a few hours in the length of time required.

The germination characteristics are quite closely connected with the life-history of the parasite. The two forms which live over within the seed produce no sporidia, while those which live over in the soil or on the kernels produce sporidia which help insure their chances of persistence.

As far as the behavior of the promycelium itself is concerned, there are differences. Fusions occurred in all except *U. zeae* and *T. foetens.* The sporidia of *T. foetens* conjugate in pairs and send out germ threads from these fused pairs. In all other forms germ threads were sent out either by the promycelium or by sporidia, when these occurred. The promycelia very commonly became detached, and free segments formed in *U. zeae, U. hordei,* and *U. avenae.* This occurred sometimes in the case of *U. tritici* promycelia growing in a nutrient solution, but was never observed in *U. nuda* or *T. foetens.*

The spores of all forms were exposed to winter conditions in Minnesota and all germinated very readily in the spring, thus demonstrating that smuts may survive the winter in the spore form under field conditions. Spores of *U. zeae* and *T. foetens* (also *T. tritici*) germinate only with difficulty when fresh, but after a resting period of a few months they germinate readily. However, fresh spores of *U. zeae* germinated in 1913. Such influence of a resting period is to be expected in smuts which live from one season to another chiefly in the spore form.

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# DESCRIPTION OF PLATES

PLATE I. Spores of *Ustilago tritici* at rest and after various stages of germination in water. × 1000. *a,* Group of spores showing various shapes and sizes; *b-k,* early stages of germination in water, *i,* showing premature vacuoles; *j* and *k,* promycelium beginning to branch; *l-r,* growth after 54 hours, showing branching and beginning of germ tubes in *p* and *r.*

II. Germination of spores of *Ustilago tritici* and growth in water during 74 hours. × 1000. *a,* Promycelium showing common form of fusion; *b* and *c,* formation of germ threads as continuations of the promycelium; *d,* germ thread forming from tip of promycelium, the basal cell vacant; *e,* branched and fused promycelium with vacant tips on three of the branches; *f,* promycelium which has sent out two branched germ tubes; *g,* promycelium apparently continuing as a germ tube after branch has been sent out; *h,* two promycelia fused and continuing as a single tube; *i,* two germ tubes from the same promycelium, one of which has sent out another germ tube which fused with the other original one; *j,* three germ tubes from the same promycelium, two of which fused.

III. Germination of spores of *Ustilago tritici* and growth in water during 96 hours. × 1000. *a, b, c, d, e,* Germinations of individual spores.

IV. Germination of spores of *Ustilago tritici* in 5 per cent sugar solution after 22 hours. × 1000. *a-j,* After 22 hours, *a-f,* showing various forms of promycelia; *h,* promycelium with loosely connected segments; *i,* promycelium separated from spore; *j,* cells of promycelium separated from each other; *k-o,* various forms of swollen tips of germ threads.

V. Germination of spores of *Ustilago tritici* and growth in 5 per cent sugar solution during ten days. × 1200. *a-g,* Various forms of promycelia and germ tubes.

VI. Germination of spores of *Ustilago tritici* and growth in 5 per cent sugar solution after 25 to 36 days. × 1500. *a-g,* Tips of germ tubes showing tendency, especially in *e* to round up into segments —25 days; *h, i,* some of the segments freed—27 days; *j,* free segments, some of which show formation of a wall—28 days; *k,* free, or resting segments which have surrounded themselves by a wall; *l,* resting segments germinating at 30 to 36 days.

VII. Germination of spores of *Ustilago tritici,* which had been frozen, and growth in distilled water during 23 hours. × 1000. *a-j,* Germinations of individual spores.

VIII. Spores of *Ustilago nuda* at rest and at various stages of germination and growth in water and 2½ per cent sugar solution. *a,* Group of spores of various shapes; *b-f,* promycelia in water at 24 hours; *e,* a typical form; *i-k,* growth in 2½ per cent sugar at 5 days, showing fusions and long, unusual germ threads.

IX. Germination of spores of *Ustilago nuda* and growth in 2½ per cent sugar solution during seven days. × 1200. *a,* Germ tube growing out from promycelium, the two end cells of which have become vacant; tip branched and fused; *b,* a fairly common form at 7 days; *c,* a simple form of young germ thread at 7 days.

X. Germination of spores of *Ustilago nuda* and growth in 2½ per cent sugar solution during nine days. × 1200. *a-f,* Germination of individual spores; *b,* a much branched form with a number of fusions; *b'* and *b''* later fused also and sent out a long germ thread from the fused portion.

XI. Germination of spores of *Ustilago nuda* and growth in 2½ per cent sugar solution during 16 days. × 1200. *a-c,* Various forms of germ tubes; *d,* typical, wavy tip of single germ tube.

XII. Germination of spores of *Ustilago nuda* and growth in 2½ per cent sugar solution during 26 days. × 1400. *a-g,* Various forms of tips of germ tubes as finally developed; fusions shown in *e* and *g.*

XIII. Germination of spores of *Ustilago nuda,* which had been kept outside from December 10 to April 8, and growth in 5 per cent sugar solution after 24 and 27 hours. × 1200. *a* to *e* at 24 hours; *f* to *i* at 27 hours.

XIV. Germination of spores of *Ustilago hordei* in water. × 1200. *a-e,* at 18½ hours; *c* and *d* show beginning of formation of sporidia; *f-m* at 21 hours; *h, l, m,* show formation of sporidia; *h* shows sterigma which sometimes appears in the formation of sporidia.

XV. Germination of spores of *Ustilago hordei* and growth in a 1 per cent sugar solution during various lengths of time. *a-d,* Early stages of germination, showing formation of sporidia; *e, f,* various forms of sporidia; *h,* a spore which has sent out two promycelia both of which are producing sporidia vigorously after 18 hours; *i,* a less usual form in which sporidia remain attached while forming other sporidia; *g,* sporidia germinating; *j,* detached sporidia budding; *k,* sporidium sending out long, narrow tube; *l,* sporidium of large size, budding from both ends at 92 hours; *m,* sporidia germinating at 4 days; *n,* two unusual sporidia, one of which is germinating while the other is budding; *o* and *p,* sporidia at 5 days; unusual shaped tube in *o; p* showing germination from one end and formation of sporidium from the other; *q,* typical promycelium at 34 hours; *r,* promycelium abjointing sporidia from tip only, at 92 hours; *s,* germination of sporidium at 5 days; *t,* germination of sporidium at 8 days; *u,* formation of sporidia at 5 days; the one at the tip germinating *in situ; v,* a resting segment; *w,* typical germ thread sent out by a sporidium, 9 days.

XVI. Germination of spores of *Ustilago hordei* and growth in 1 per cent sugar solution, for various lengths of time, illustrating the development of germ tubes. × 1200. *a-e,* Various stages in development of germ tubes at 48 hours; *f,* two tubes from same spore, fused and branched, 8 days; *g,* detached promycelium sending out germ thread at 12 days; *h,* an unusual form with apparently no septations in vacant portions, 94 hours; *i,* two germ threads from

same promycelium at 9 days; *j,* two promycelia from same spore, one sending out a germ thread at 12 days which is unusually short.

XVII. Germination of spores of *Ustilago hordei* and growth in 1 per cent sugar solution after from nine to twelve days. × 1400. *a-e,* Various forms in 1 per cent sugar solution at nine and twelve days; *a* and *b,* tips of germ threads with characteristic fusions; *c,* tip of germ thread showing tendency to round up into segments; *d,* sporidium producing germ tube; *e,* very thick promycelium which has sent out no germ threads; *f-q,* forms at 12 days; *g, i, o, p, q,* and *r,* show resumption of activity by resting segments and sporidia after culture had been dried out; *q,* shows an almost globose sporidium, which rounded up and became rich in oil content, beginning to germinate; *i,* shows a resting sporidium like that shown in *q* sending out a tube appearing much like the original promycelium.

XVIII. Germination of spores of *Ustilago avenae* and growth in water during various lengths of time. × 1200. *a-f,* Early stages of germination showing typical promycelia with formation of sporidia in *c, d, e,* and *f; g,* a "knee joint" fusion, which, due to the growth of the promycelium, has opened up; *h,* development of two promycelia from the same spore; *i,* production of sporidia from septa and tip; sterigma at septum; *j,* non-septate promycelium; *k,* promycelium in which vacuoles are appearing; *l,* germ tube being sent out from basal segment; *m-r,* various forms at 32 hours; *s-v,* forms at 60 days; *t-v,* showing free segments; *w,* tip of germ tube at 13 days; *x,* germ tube at 13 days budding sporidia from portions filled with protoplasm.

XIX. Germination of spores of *Ustilago avenae* and growth in sugar solution during various lengths of time. × 1200. *a-n,* Various forms of promycelia at 24 hours; *o,* detached sporidia budding; *p,* branched promycelium producing sporidia; *q, r,* germinating sporidia; *s,* promycelia producing germ tubes which have fused at 11 days; *t,* germ tube at 7 days.

XX. Germination of spores of *Ustilago zeae* and growth in water and manure decoction during various lengths of time. × 1200. *a-f,* Germination in manure decoction at 19 hours; *g,* formation of germ tube in water at two days; *h* and *i,* promycelia at 7 days producing sporidia; *j, k,* typical sporidia; three lower in *k* germinating; *l,* detached sporidium which has germinated from both ends and become vacant as a result; *m* and *o,* free segments budding; *n,* sporidia, some of which are budding; *p,* sporidia sending out germ threads in water in 4 days; two sporidia have lost protoplasm after germination; *q,* chain of sporidia in manure decoction at 44 hours; *r-t,* detached segments sending out germ threads; *s,* detached segments budding; *u,* promycelium sending out germ tube.

XXI. Germination of spores of *Ustilago zeae* and growth in 1 per cent sugar solution during 27 days. × 1400. *a,* Germinating sporidia; *b,* ungerminated sporidia which have become rich in oil; *c,* in-

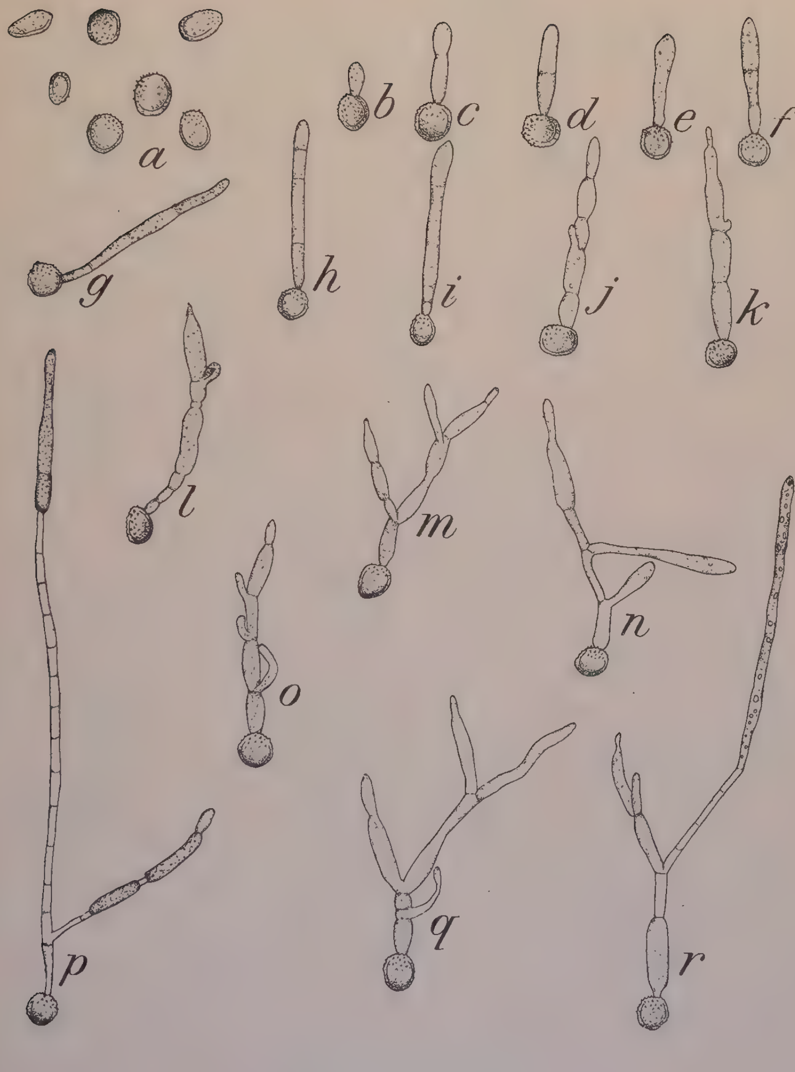
dividual promycelial cells sending out germ tubes from both ends; *d*, promycelium sending out a number of germ threads; *e*, attached promycelia which have sent out no germ tubes and have large oil globules; *g*, detached promycelial cells sending out tubes; the one to the left has two air conidia; *h*, promycelial segments sending out long threads.

XXII. Germination of spores of *Ustilago zeae* and growth in 1 per cent sugar solution during 27 days, showing formation of much-branched chains of air conidia which are usually fairly thick-walled and rich in oil globules. $\times$ 1200.

XXIII. Germination of *Tilletia foetens* in water after 5 days, showing typical spores with many oil globules and the split epispore out of which the promycelium comes, also typical forms of germ tubes. $\times$ 430.

XXIV. Germination of spores of *Tilletia foetens* and growth in water for various lengths of time. *a-s*, Various forms of promycelia at 3½ days; *t* and *u*, beginning of process of formation of sporidia at 4 days; *v*, typical whorl of sporidia, two of which have fused, 4 days; *x* and *y*, fused sporidia which have formed secondary sporidia and lost their protoplasm; *z*, whorl of sporidia, two of which have fused and sent out a narrow germ thread; *aa*, sporidia fusing after detachment; *bb*, fused sporidia which have formed a secondary sporidium, secondary sporidium sending out germ threads; *cc*, fused sporidia which have sent out a short germ tube from the tip.

XXV. Germination of spores of *Tilletia foetens* and growth in water during various lengths of time. *a-s*, Various forms of promycelia at 5 days; *b*, fused pair of sporidia with no protoplasm; *c*, abnormal promycelium at 5 days; *d*, conjugated sporidia which have sent out two branched germ tubes from the tip. The germ tubes are producing secondary sporidia; *e*, *f*, detached secondary sporidia sending out germ threads; *g*, fused sporidia sending out a germ tube which is producing a secondary sporidium; *h*, whorl of sporidia, mostly fused, sending out germ tubes directly and from secondary sporidia; *i*, formation of secondary conidia in a linear series—unusual; *j*, *k*, typical, long, narrow germ tubes sent out by fused sporidia.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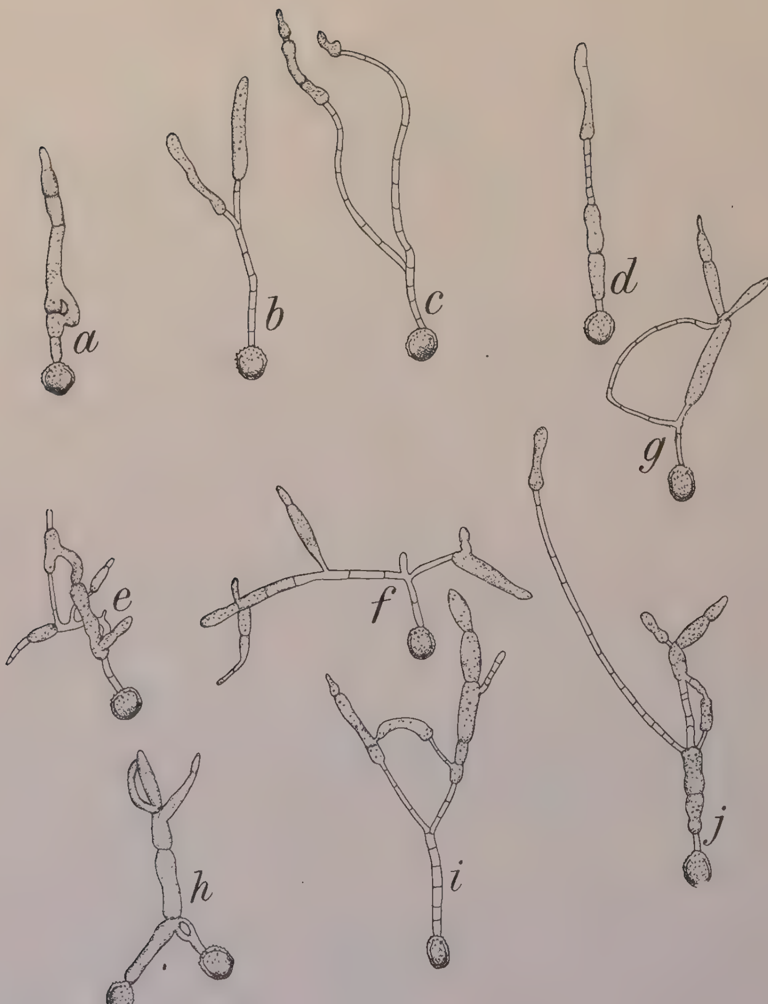
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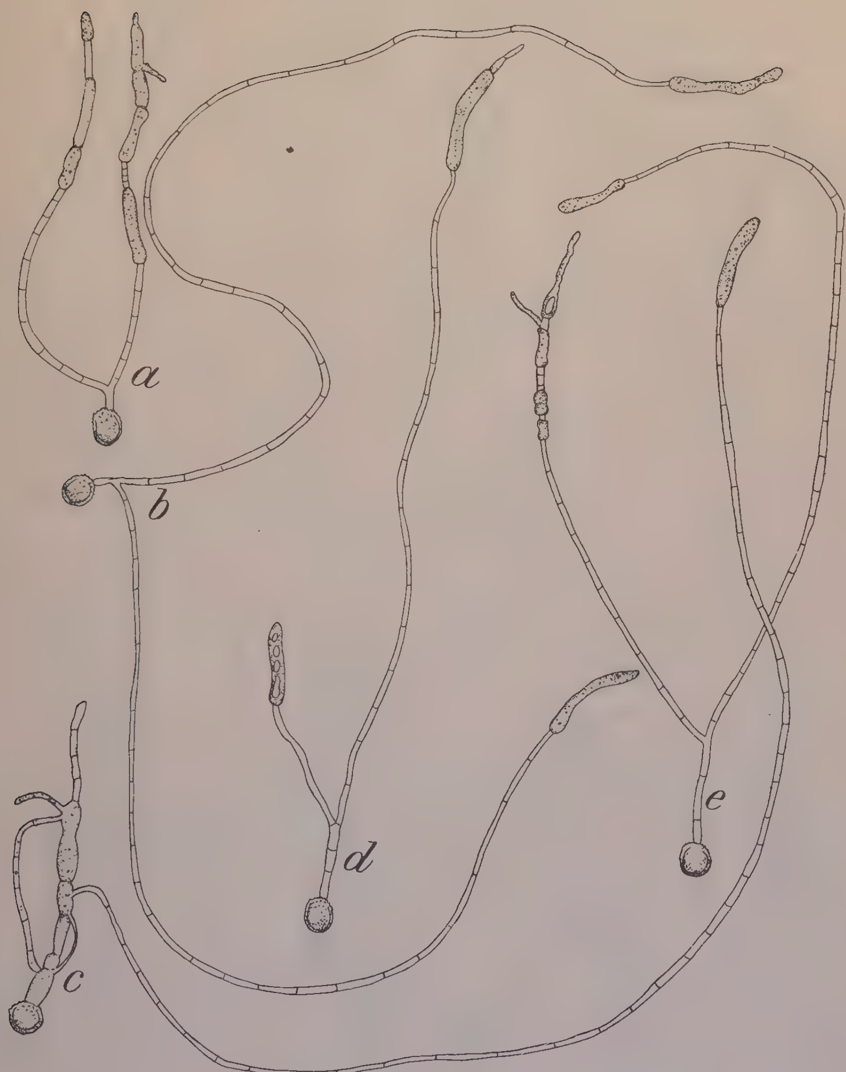
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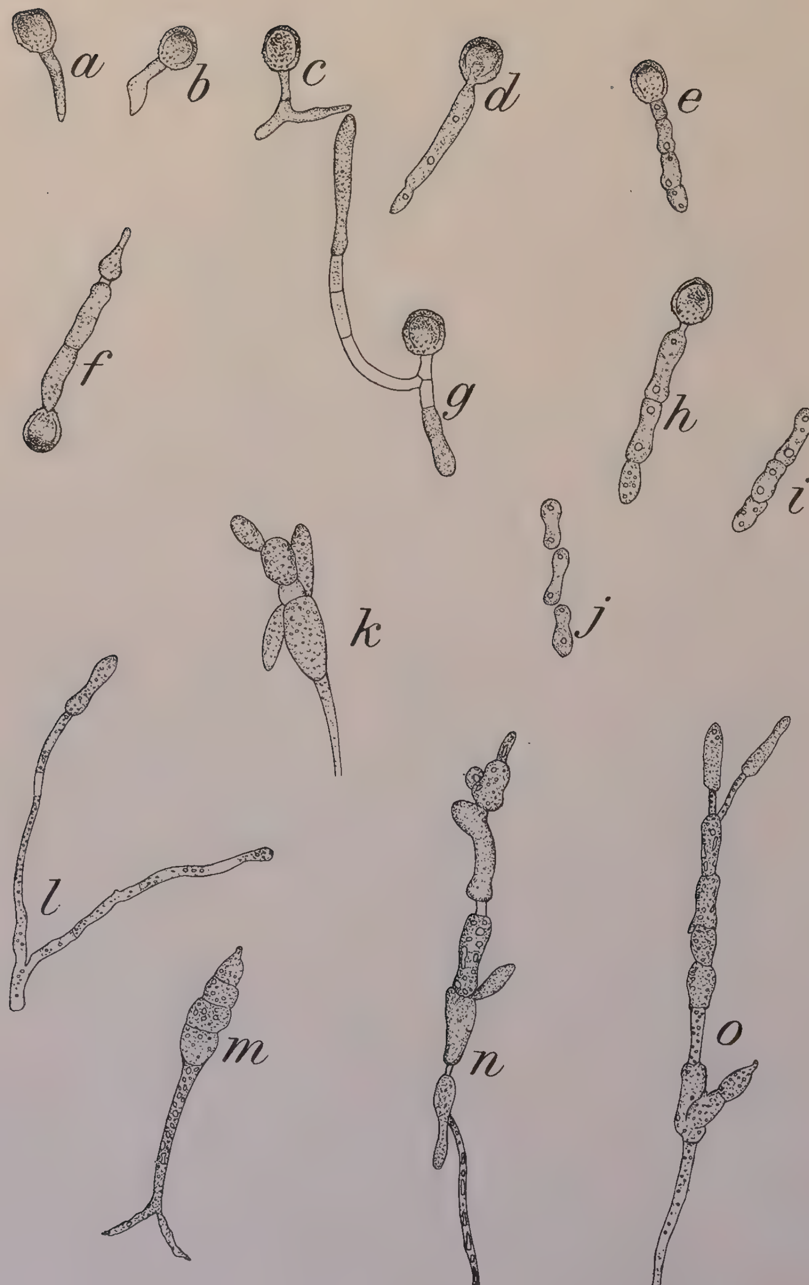
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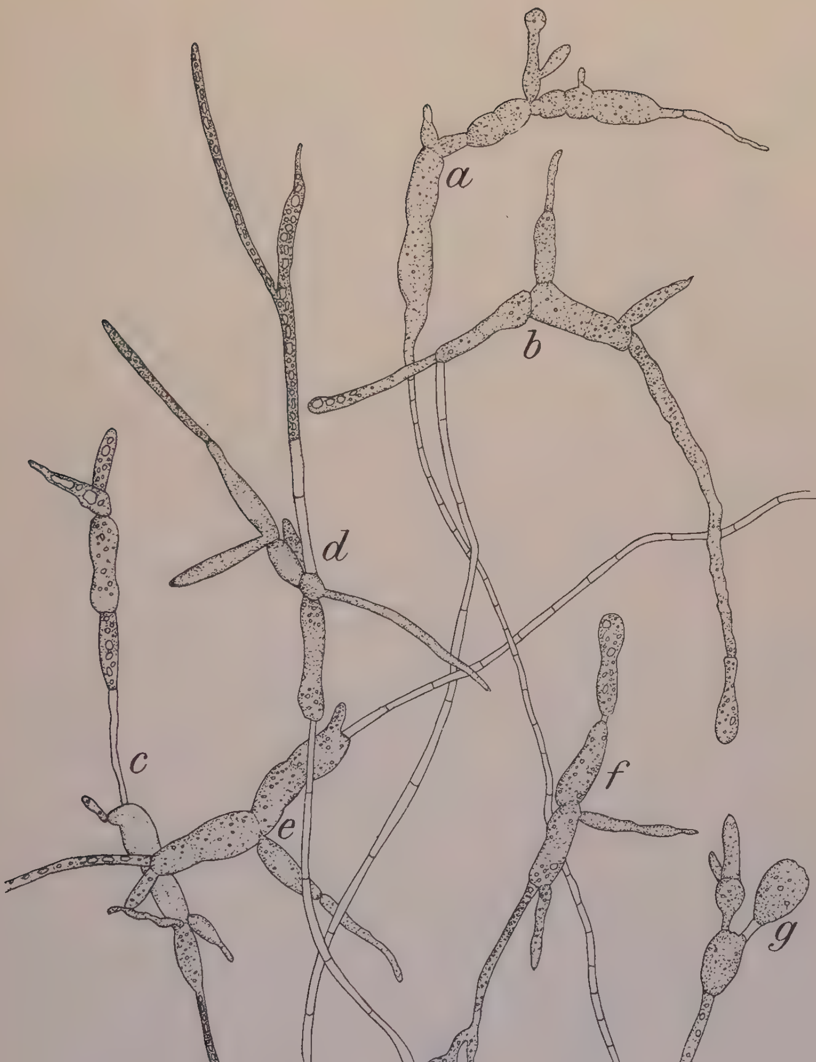
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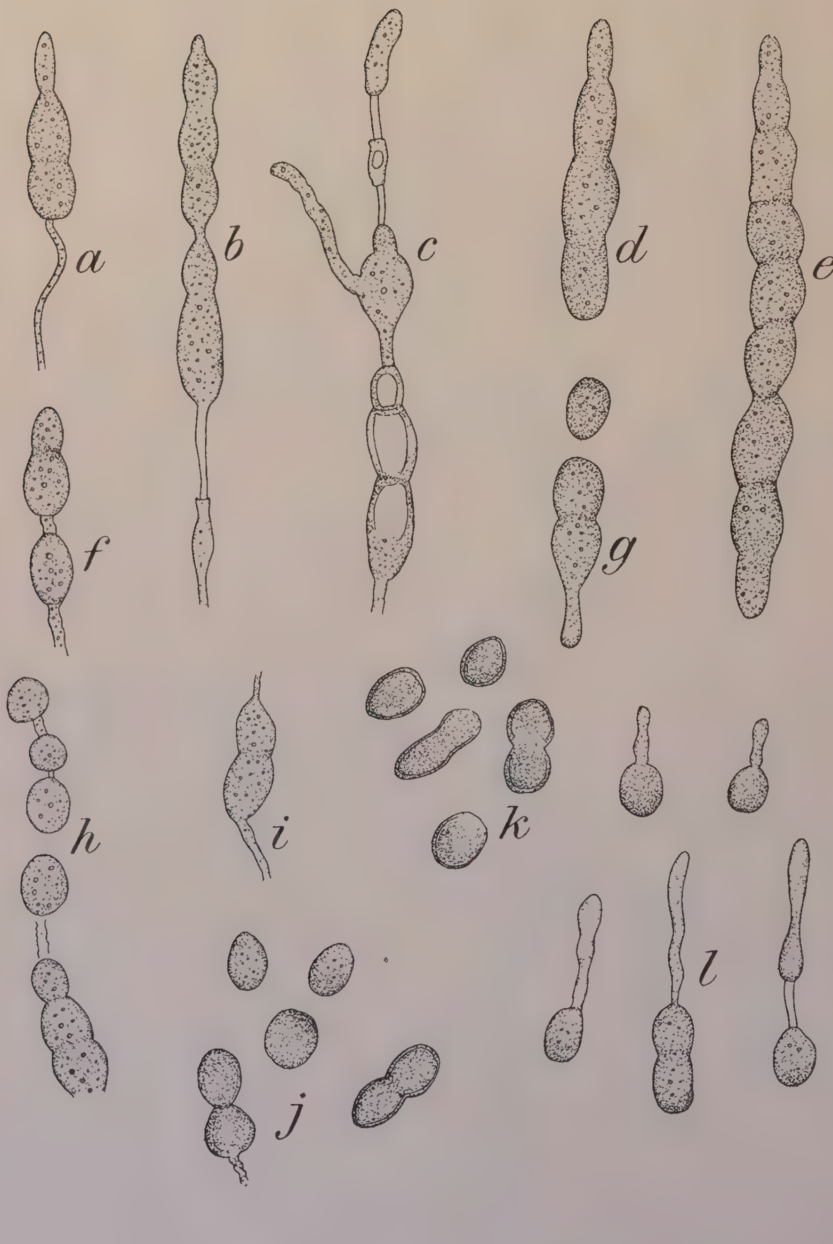
PLATE VI



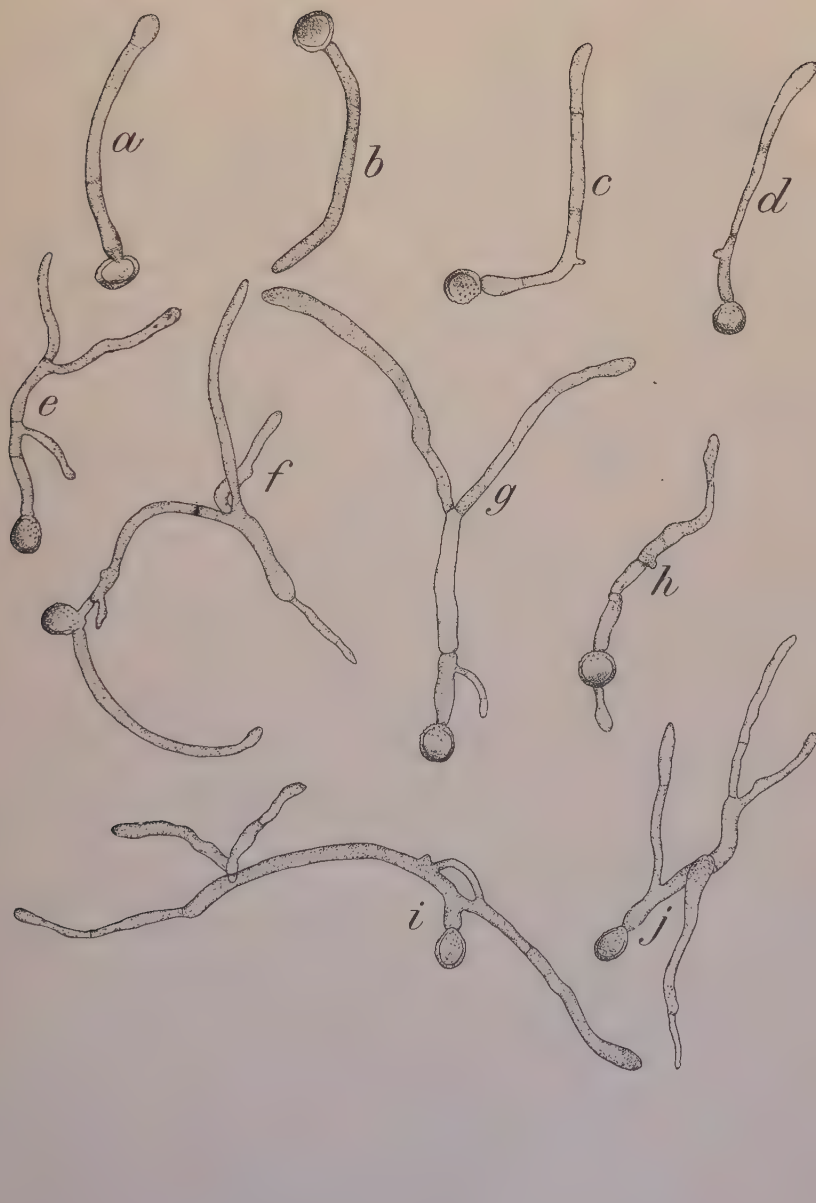

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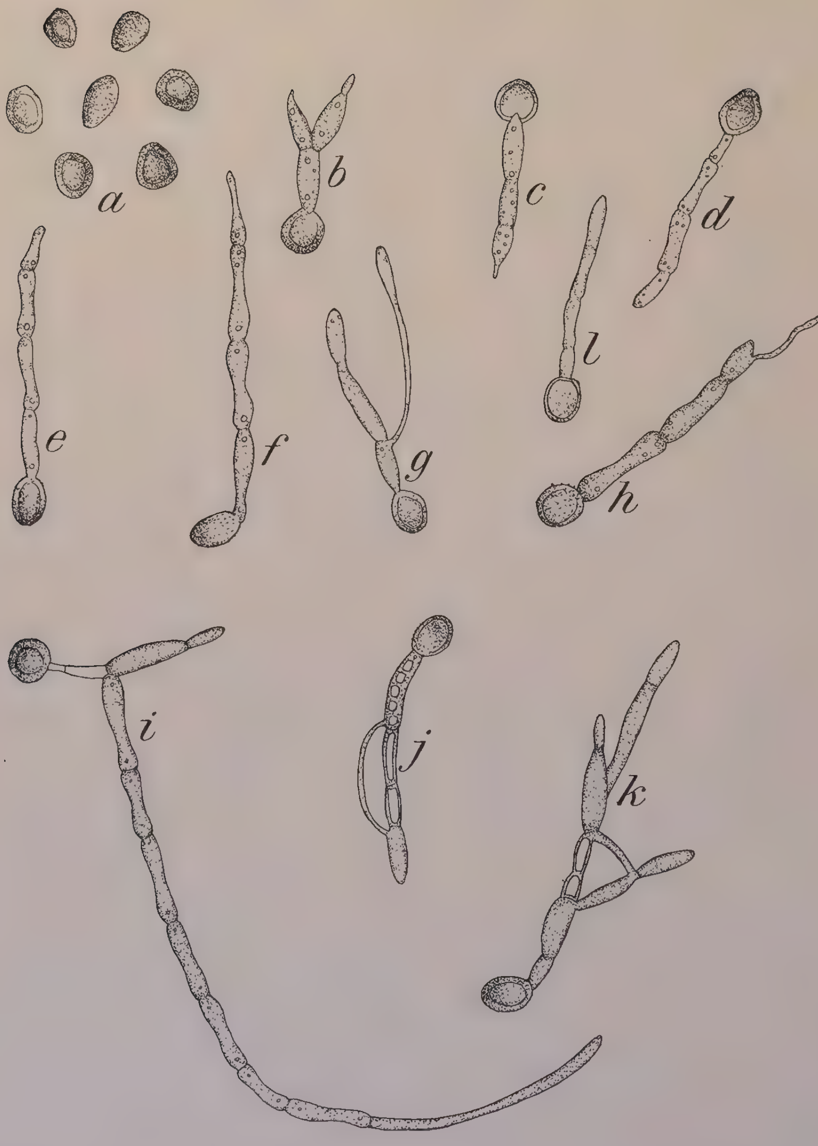
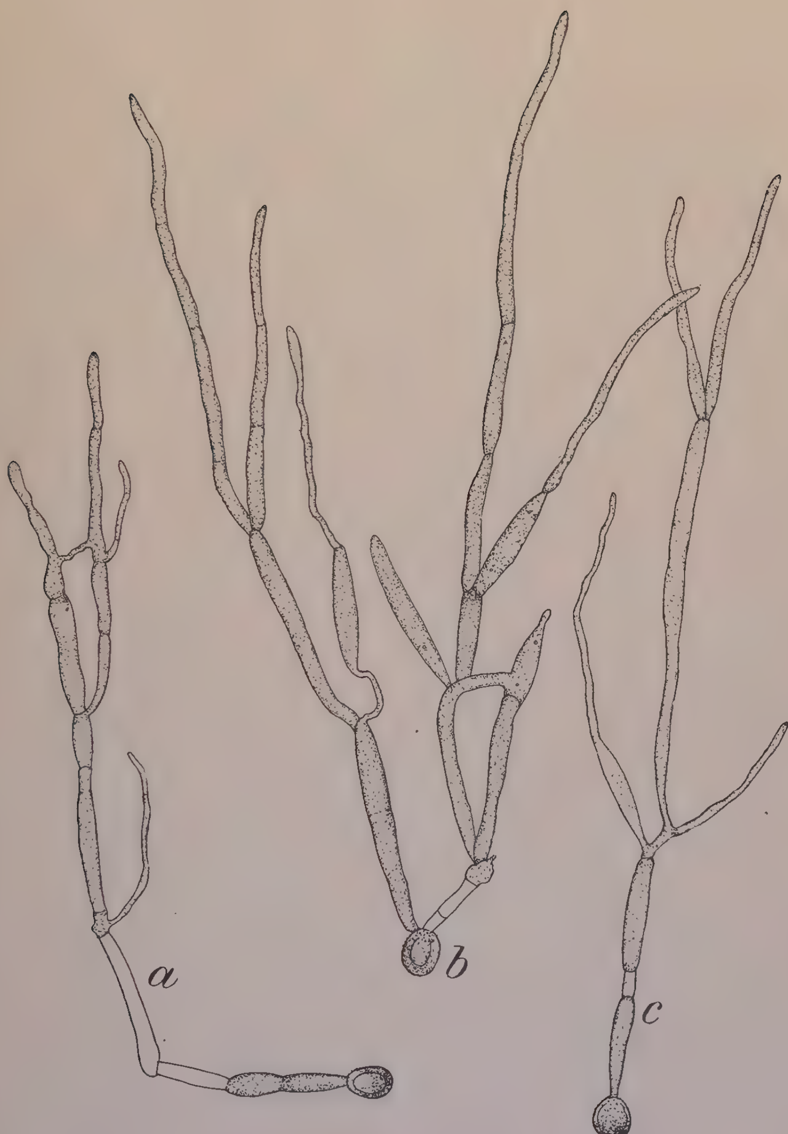
PLATE VIII

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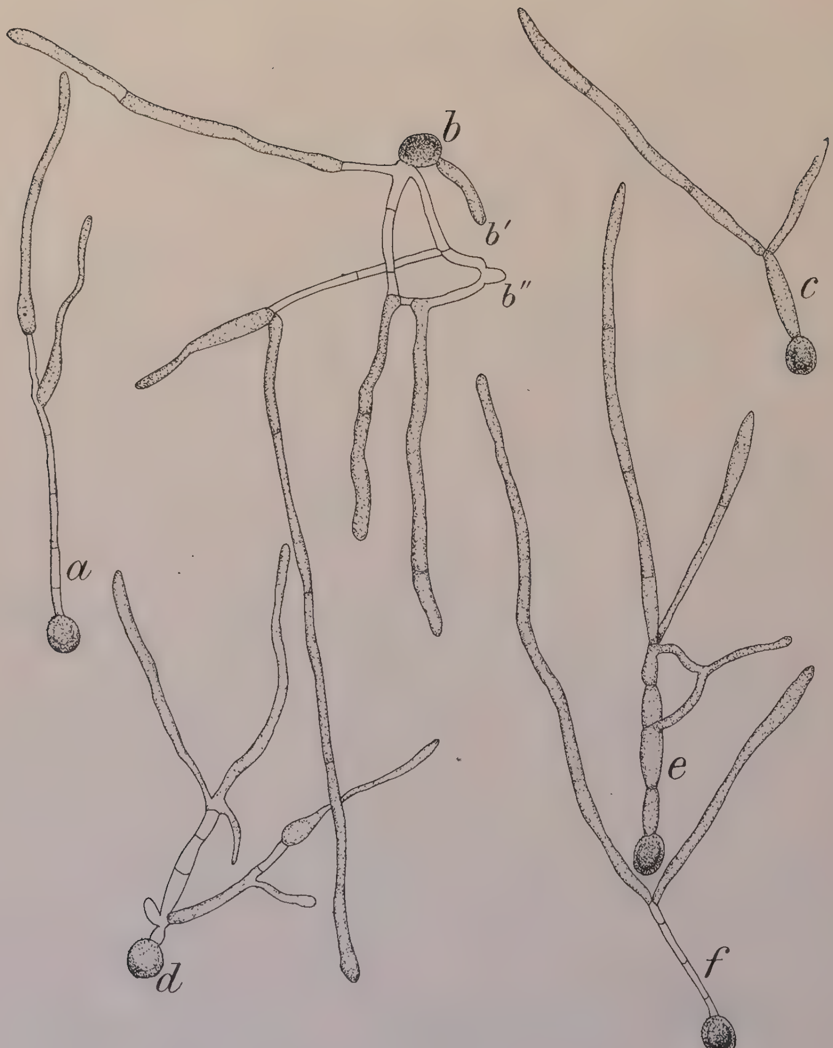
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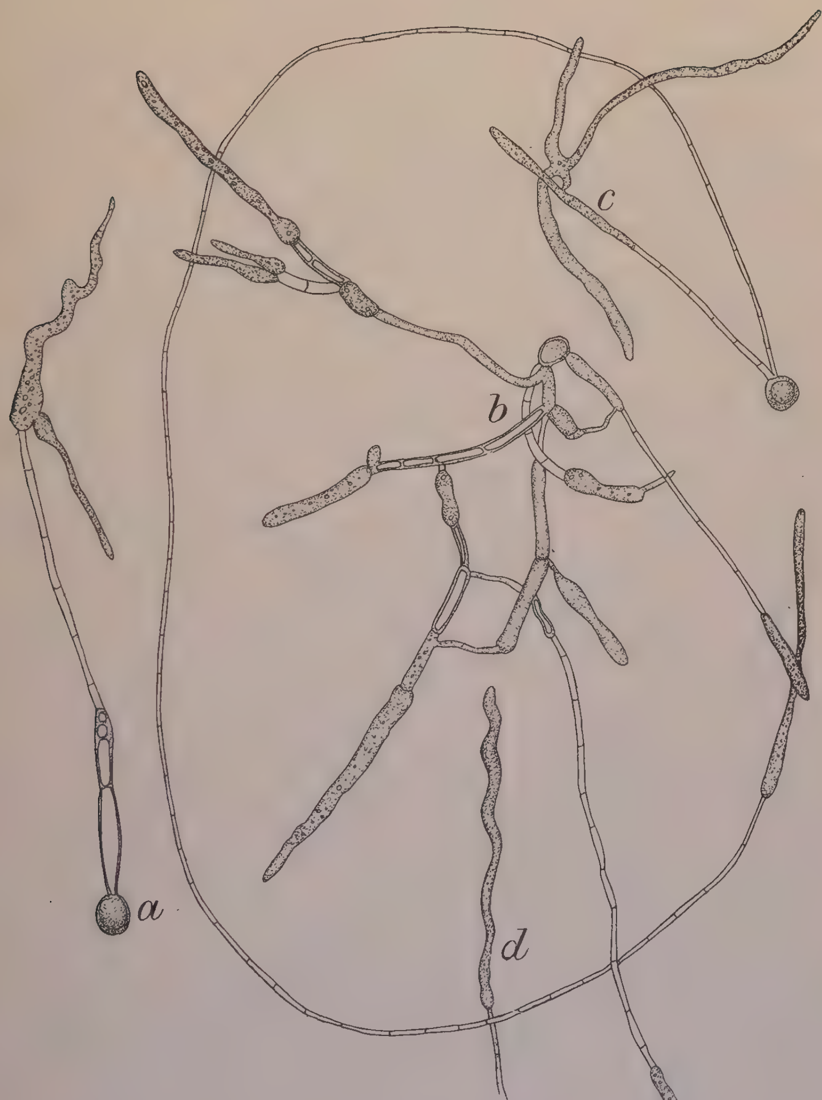
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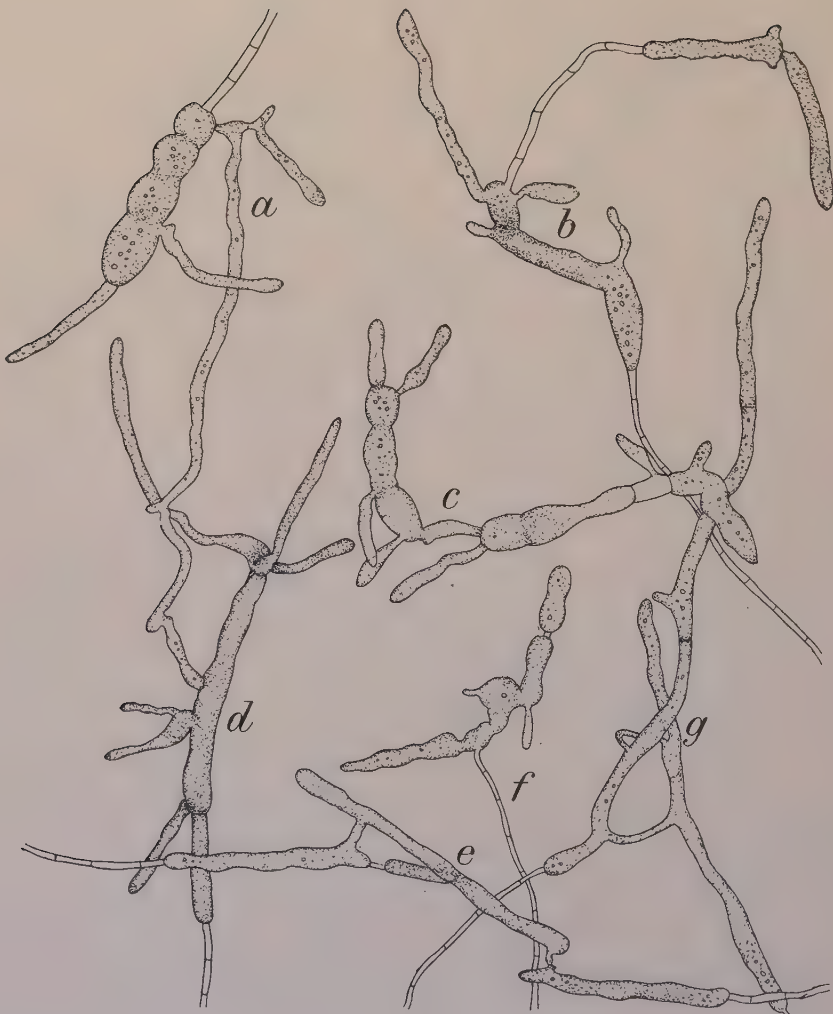

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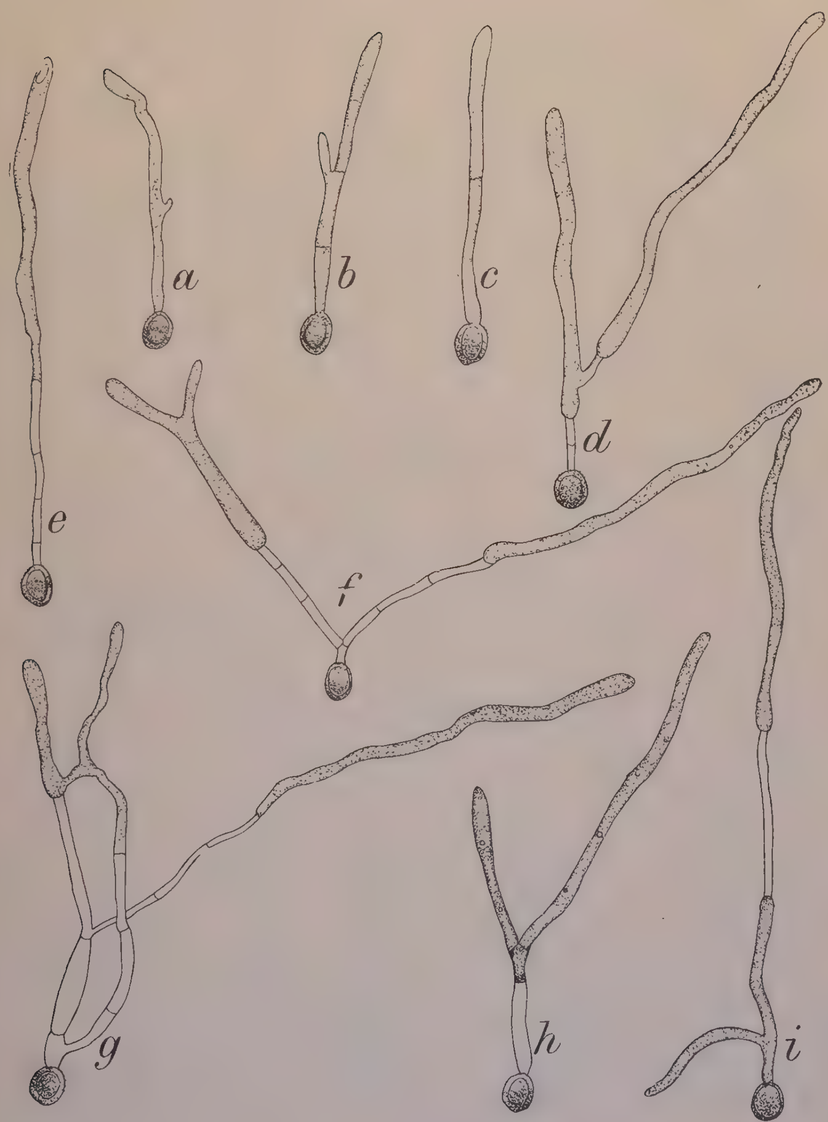
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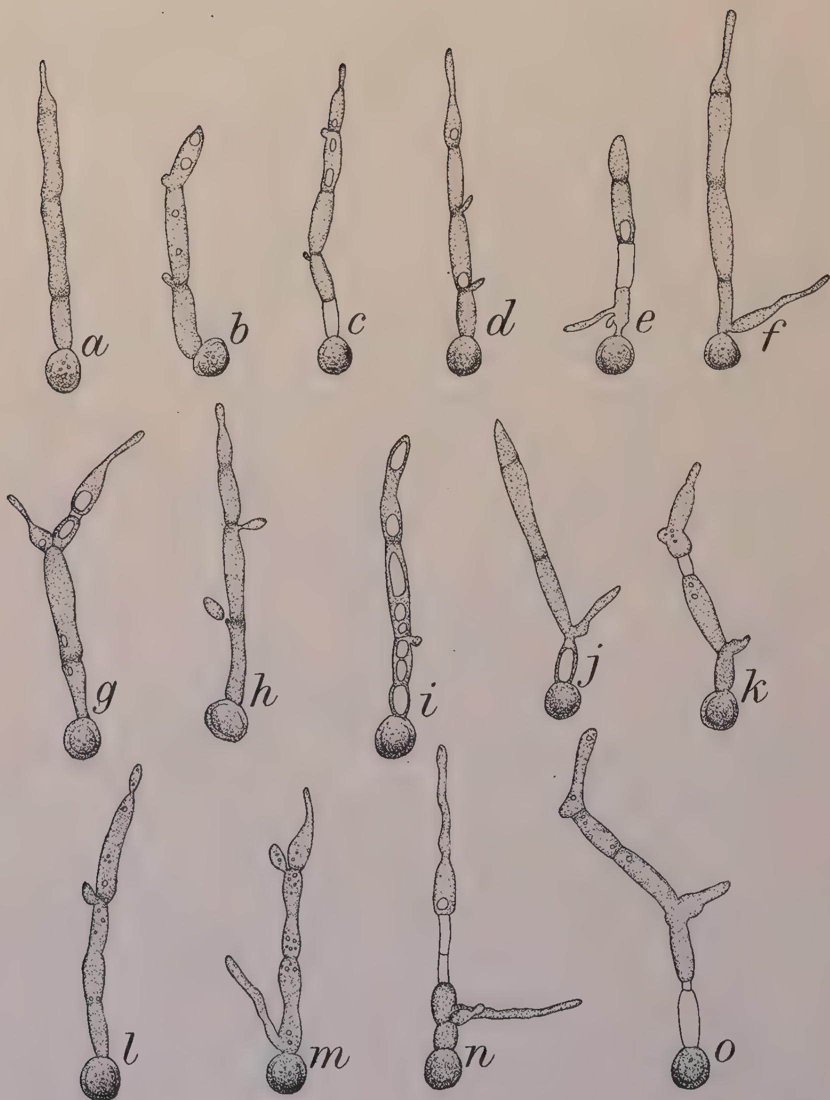
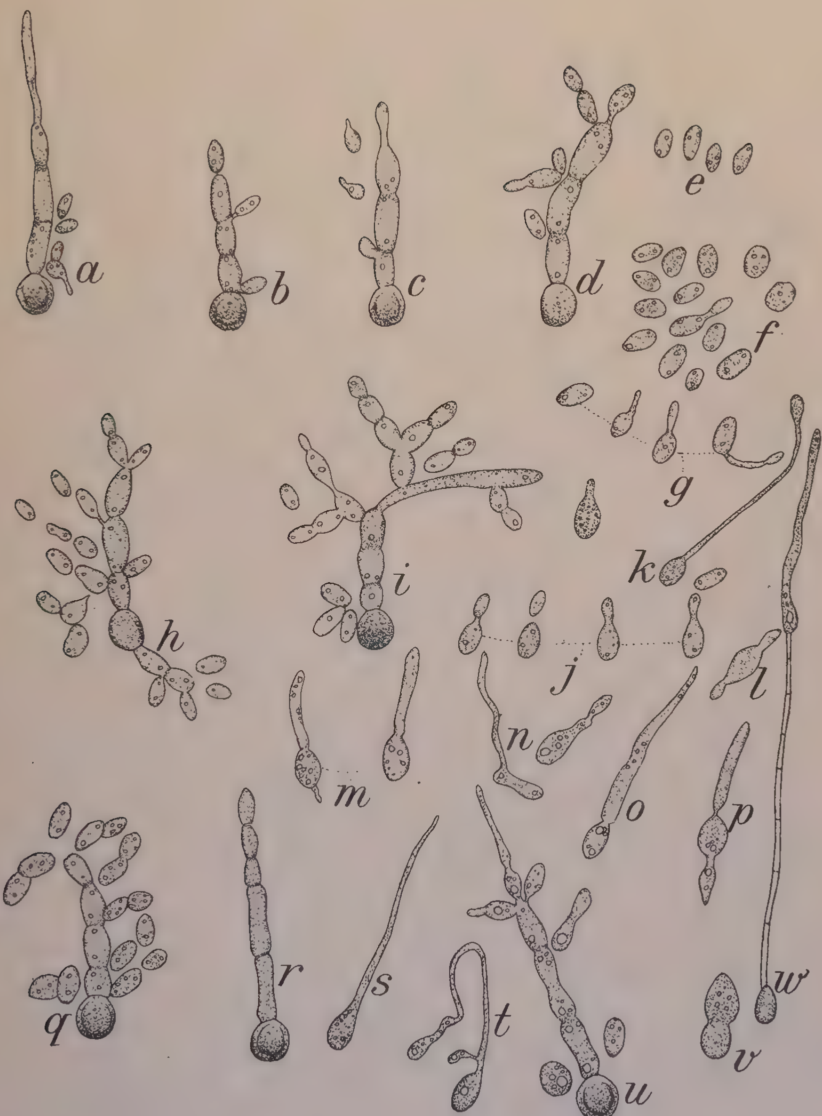
PLATE XIV

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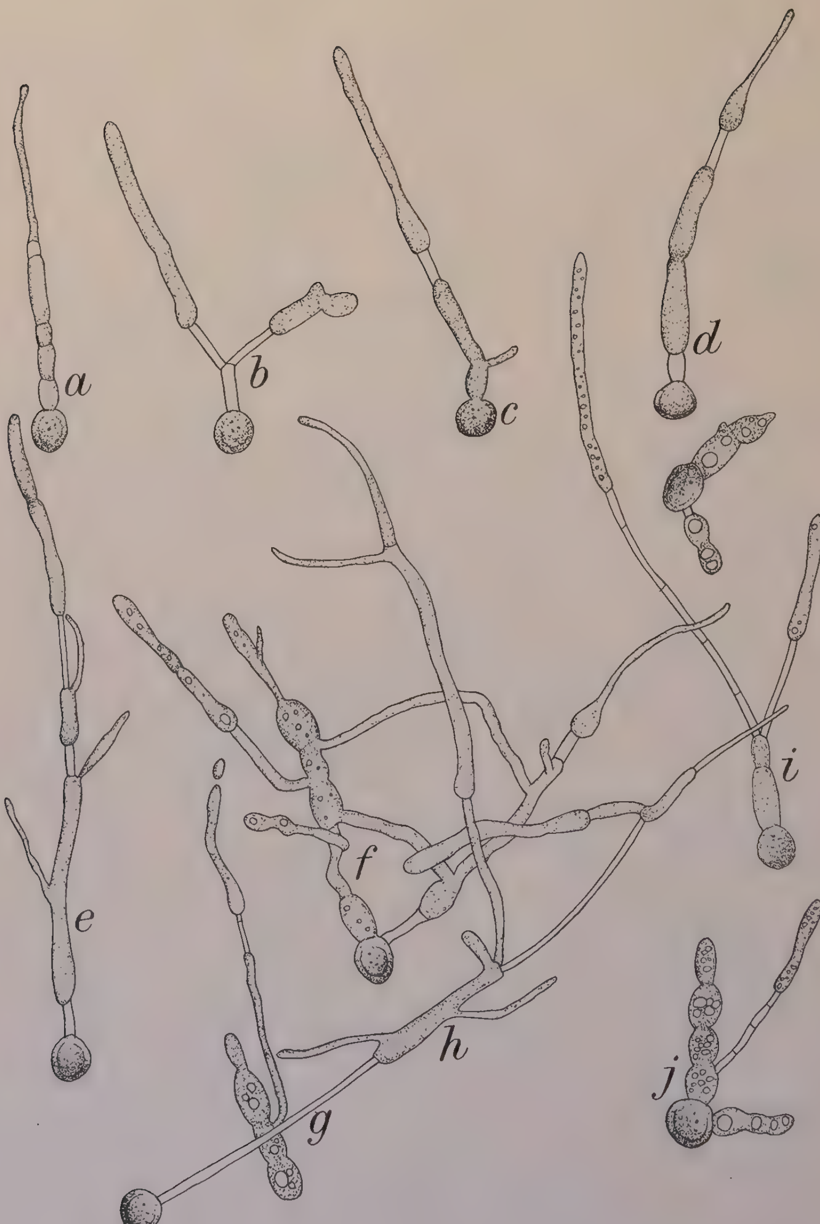
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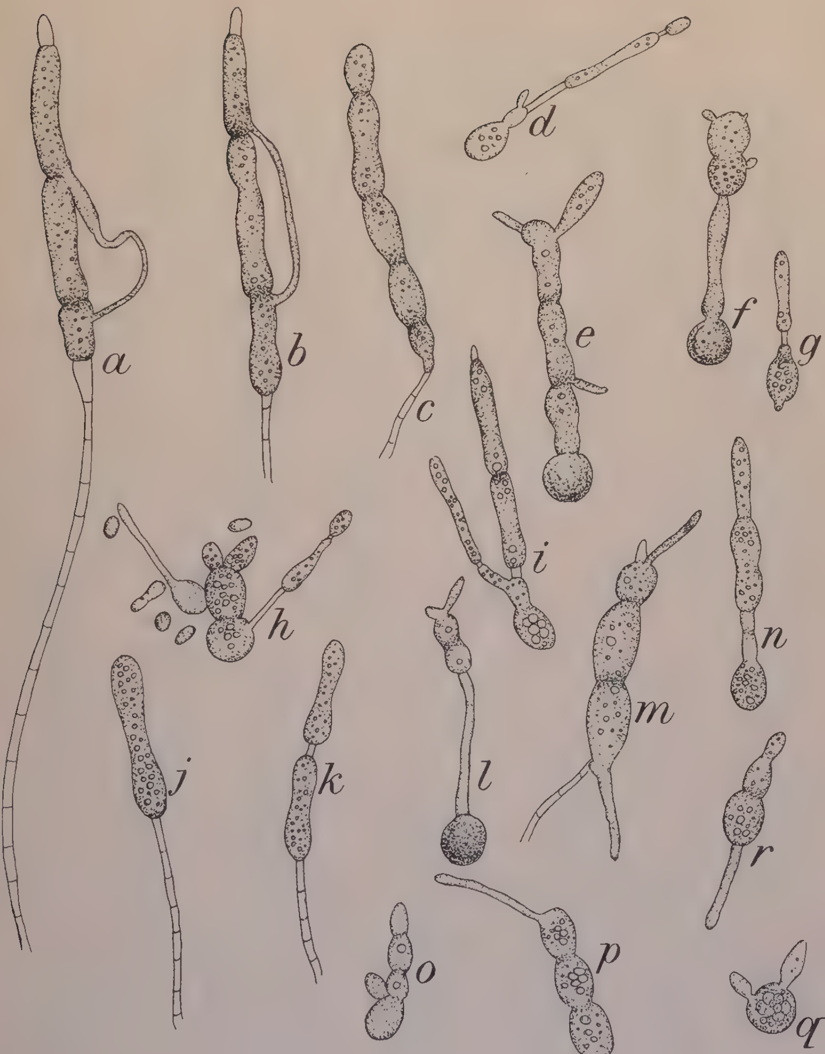
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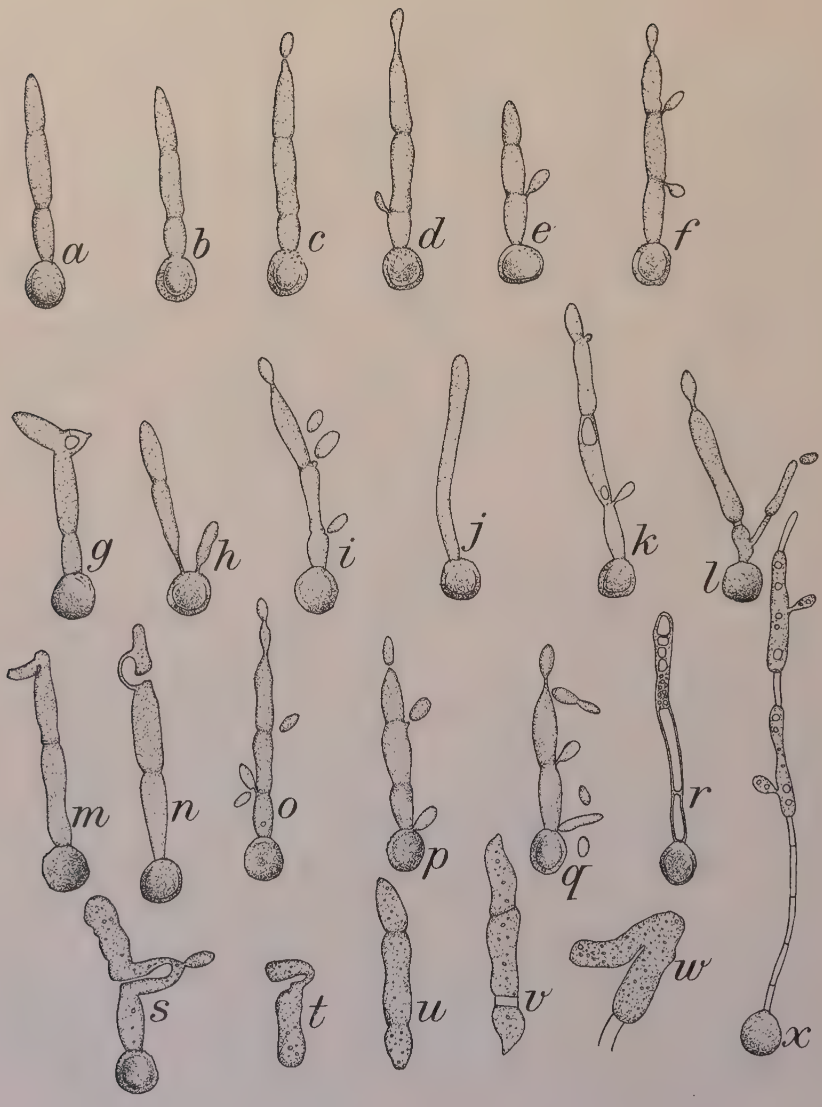
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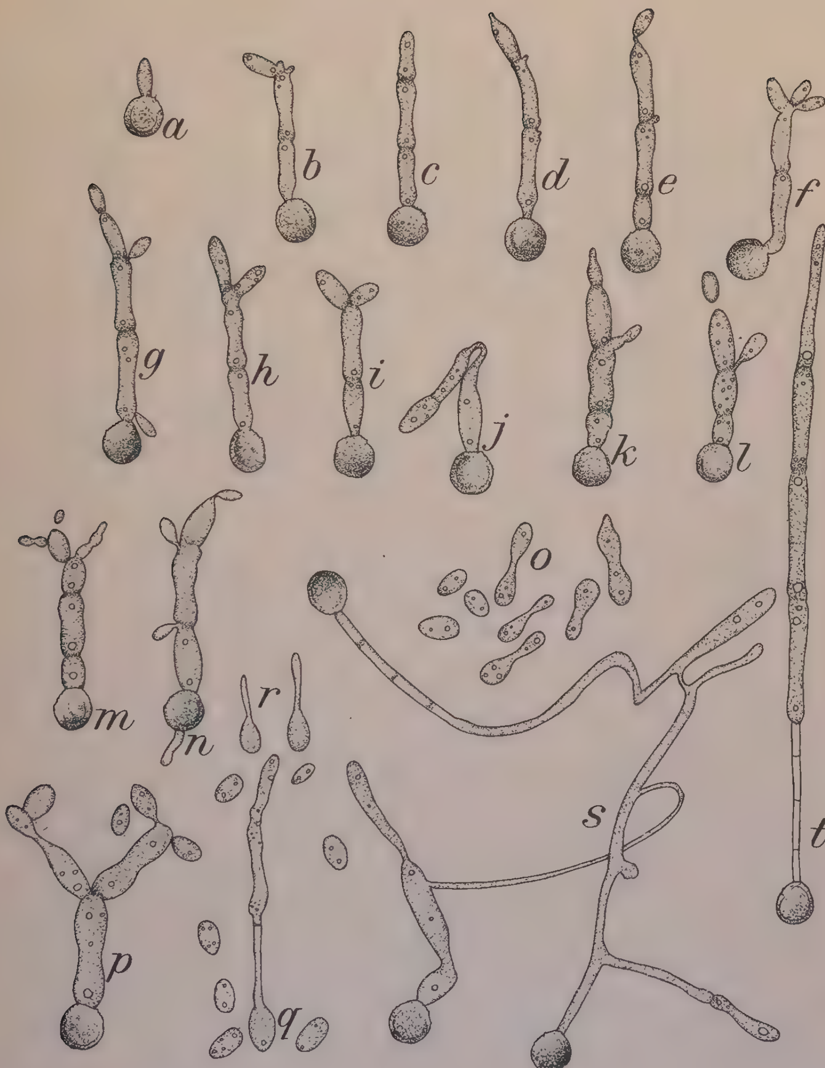
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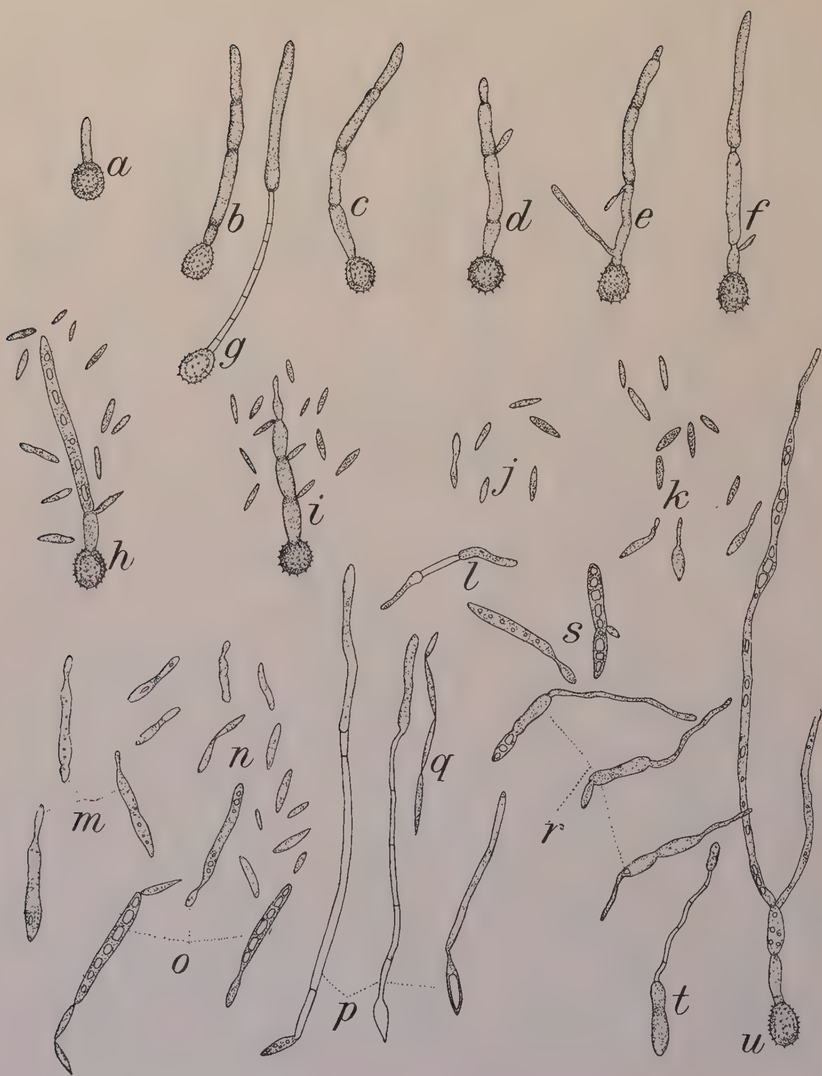
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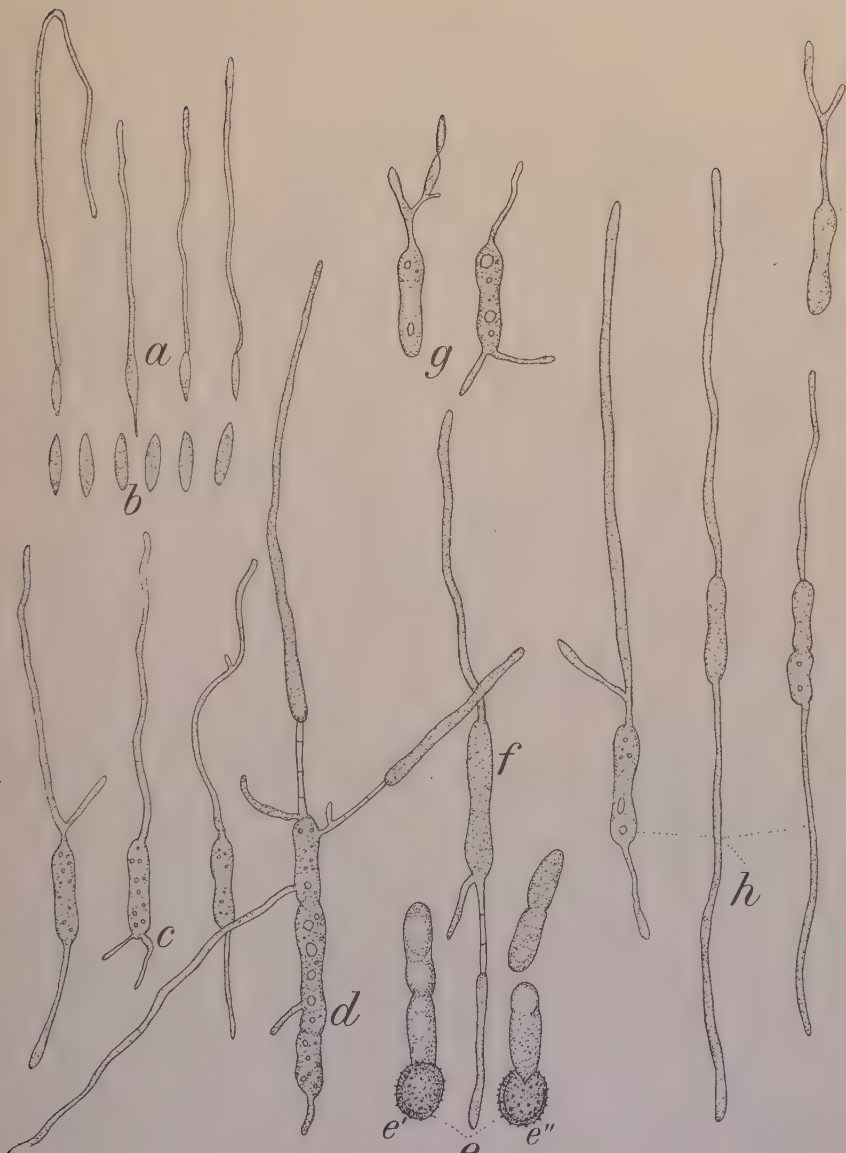
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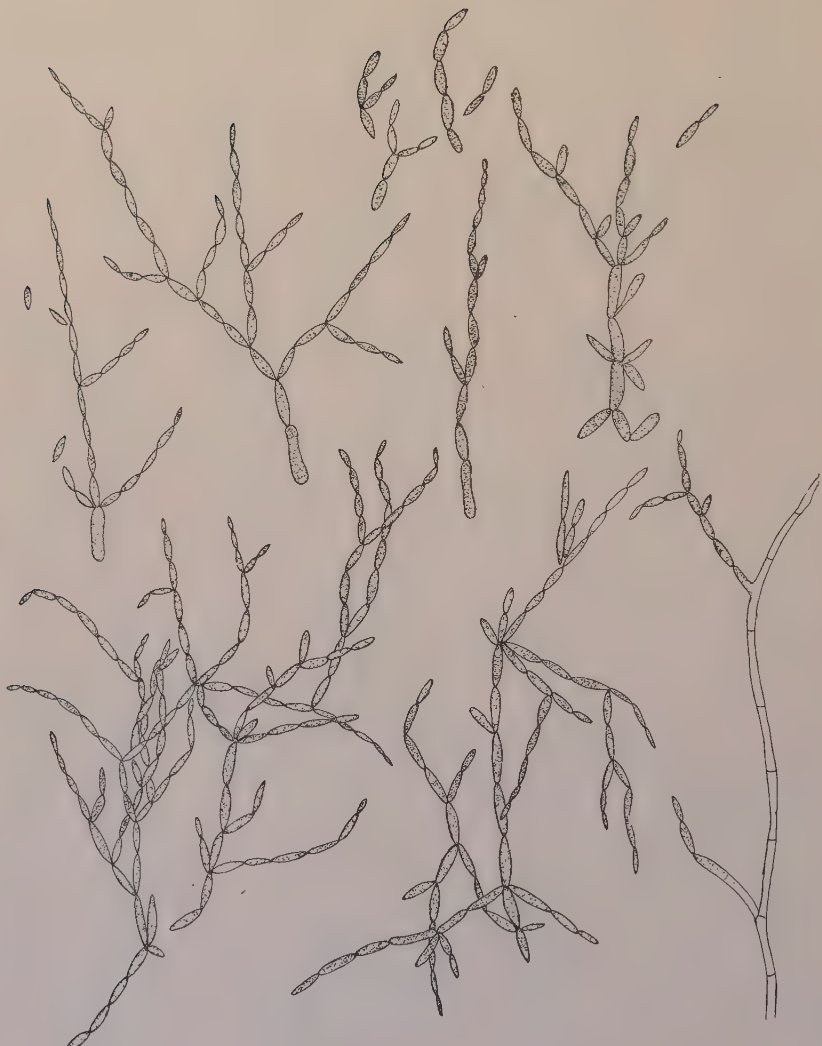

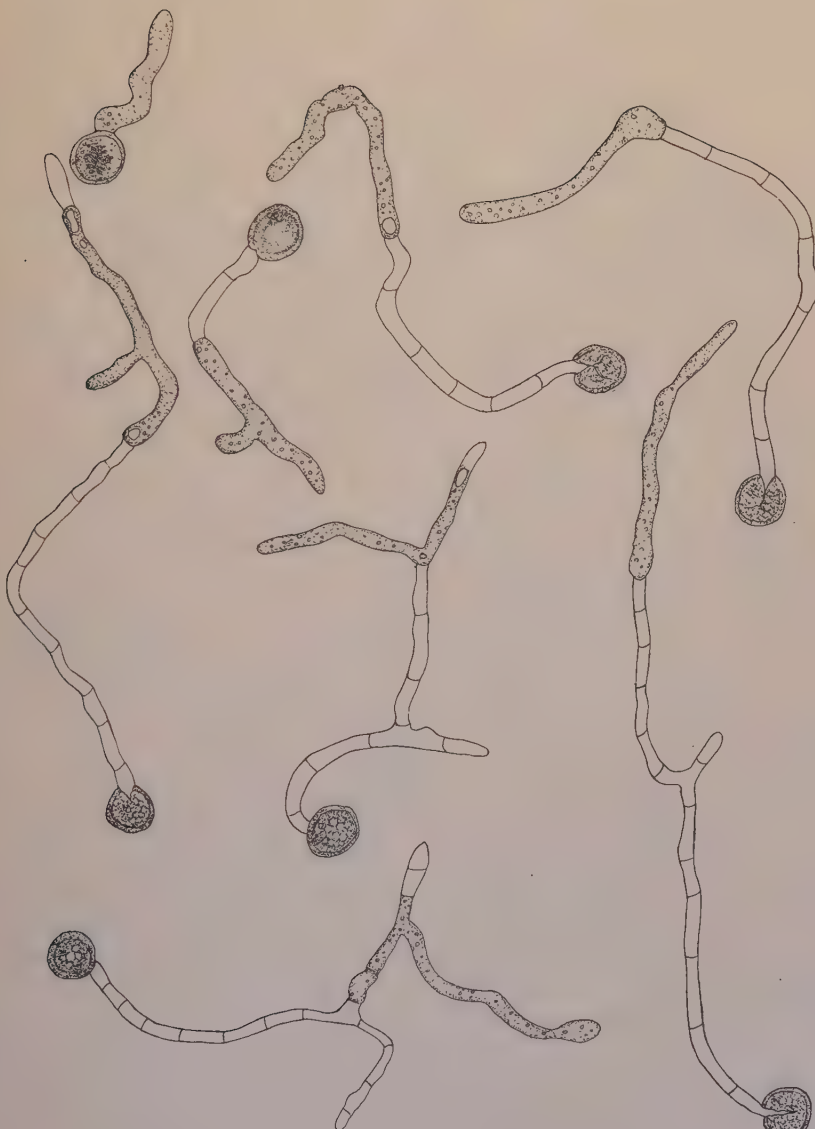

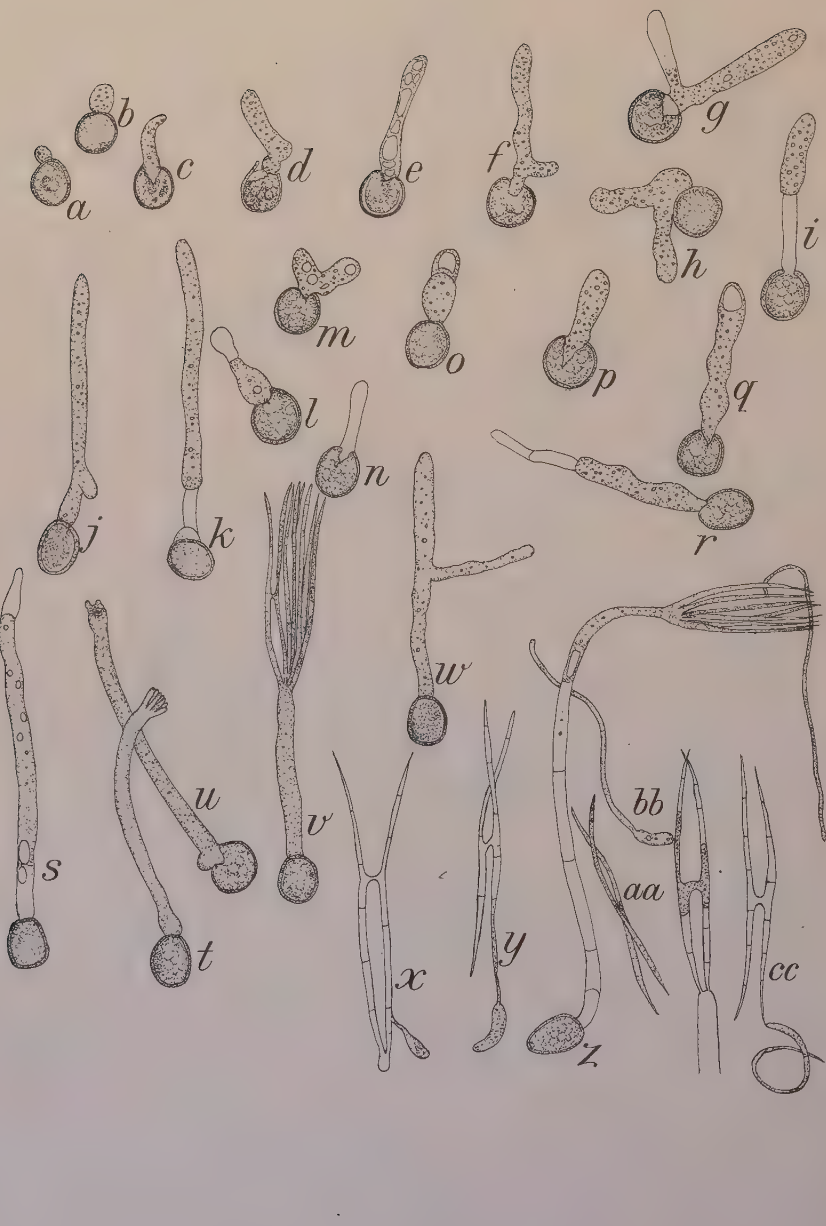
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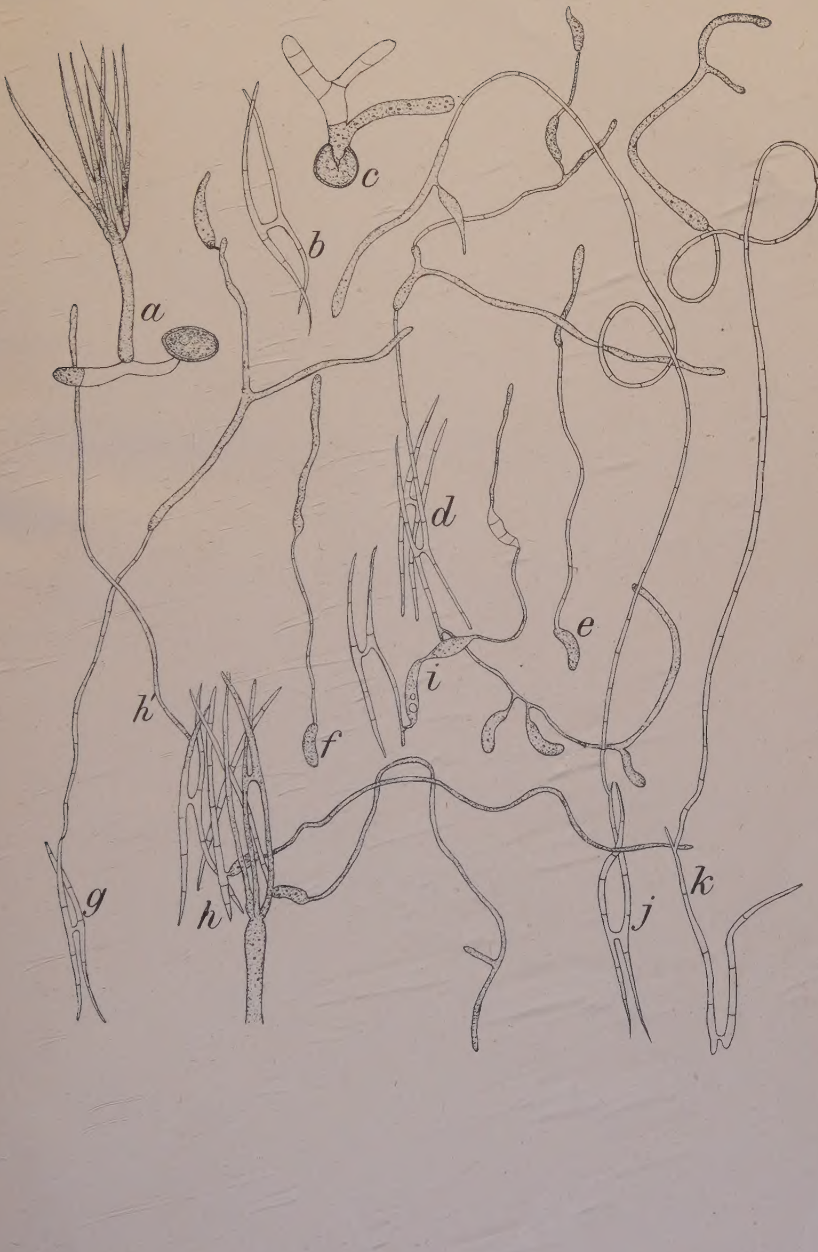
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